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METABOLIC STUDIES WITH HUMAN GROWTH HORMONE IN DWARFISM AND ACROMEGALY

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ABSTRACT

Human growth hormone failed to cause nitrogen retention in 4 primordial dwarfs. In 3 of 4 other children, there was a small but distinctly positive nitrogen balance. The primordial dwarfs were not completely refractory to human growth hormone since it caused an increase in the level of plasma unesterified fatty acid and an increase in urinary calcium excretion. In 4 adult patients with acromegaly, human growth hormone was ineffective as an anabolic agent. It did, however, cause an increase in the urinary excretion of calcium. Since 2 of the 4 subjects had clinically inactive disease, it is proposed that the tissues of these subjects had responded maximally to endogenous HGH and subsequently maintained this response. The administration of androgen resulted in nitrogen retention, indicating different sites or mechanisms of deposition of protein.

THE effectiveness of growth hormone in promoting linear growth has been demonstrated experimentally on many occasions, and more recently has been noted in man (1). In primordial dwarfism, linear growth is poor in spite of normal or only slightly decreased skeletal maturation. This has been variously interpreted as an isolated deficiency of growth hormone or as end-organ resistance to growth hormone. It seemed appropriate, therefore, to examine the metabolic effects of human growth hormone in certain growth problems, including primordial dwarfism.

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* Deceased.

In adults, abnormal growth is observed in acromegaly. There is presumptive evidence that this is a result of the excessive secretion of growth hormone. Since the nitrogen retention uniformly produced in adults by human growth hormone (HGH) is probably a response to pharmacologic amounts of the hormone (2), we considered it pertinent to test the effects of HGH in the acromegalic—who is already producing an excessive amount of HGH. The lack of anabolic effect of HGH in such patients led us to examine the effects of a different class of anabolic agents, the androgens, in patients with acromegaly. Two of the acromegalics were restudied with growth hormone following adequate x-ray therapy to the pituitary gland, at a time when there was clinical evidence of remission of the acromegaly.

MATERIAL AND METHODS

The preparations of HGH have been described in a preceding paper (2), and have been shown to cause nitrogen retention in the adult. All studies were carried out using a full metabolic balance regimen, with methods previously noted (3). Plasma unesterified fatty acid (UFA) levels were determined by the method of Gordon (4).

The plasma UFA response to HGH was established by measuring the increase in plasma UFA five hours following the intramuscular injection of 5 mg. of HGH. The results were compared with those of a control study performed on the previous day, using an injection of physiologic sodium chloride. The subjects fasted following the evening meal until after the five-hour sample of blood was drawn.

Subjects. Eight children were studied, 4 of whom were primordial dwarfs. Their pertinent features are presented in Table 1. Three of the primordial dwarfs had some craniofacial abnormalities. In Patients J.M. and M.M., idiopathic hypoglycemia had been diagnosed previously by Dr. Lawson Wilkins, and hypoglycemia was present in J.M. after a 17-hour fast. Thyroid and adrenal function, as measured by the serum levels of protein-bound iodine and cortisol, were normal in all of the subjects. Of the other 4 children, 1 was normal, and 3 had various disorders, as listed in Table 1.

TABLE 1. DESCRIPTION OF THE CHILDREN

Patient	Sex	Chronologic age (yrs.)	Height age (yrs.)	Bone age (yrs.)	I.Q.	Thyroid function	Adrenal function	Diagnosis
M.M.	M	6½	3½	6	60	N*	N	Primordial dwarfism; history of idiopathic hypoglycemia
J.M.	F	6	1½	6	84	N	N	Primordial dwarfism; idiopathic hypoglycemia
D.G.	M	17	8	16	118	N	N	Primordial dwarfism
J.H.	M	10	5	8	115	N	N	Primordial dwarfism
Car.K.	F	13	8	12	†	N	N	Gonadal dysgenesis
Cat.K.	F	13	15	13	†	N	N	Normal
J.W.	M	10	6	—	†	N	N	Achondroplasia
P.B.	M	15	12	14	†	N	N	Agammaglobulinemia

* N = normal.
† Not tested, but apparently normal.

Four adult acromegalics were studied. Two of them had not been treated, and clinically had active disease. Patient J.D. was a 48-year-old woman with a history of acromegaly for six to eight years. Following her first study, she received x-ray therapy (4,000 r) to the pituitary gland. The second study was performed six months later. At this time there had been a decrease in the size of the tongue and the hands. Patient M.M. was a 57-year-old woman with acromegaly of about ten years' duration. Following her first study, she received x-ray therapy (4,000 r) to the pituitary gland. Her second study was performed ten months after the first. At this time there was minimal evidence of subsidence of activity of the acromegaly. Patient G.W. was a 55-year-old man with questionably active acromegaly who had been treated by radiation six years earlier. Patient O.W. was a 69-year-old man with inactive acromegaly following x-ray therapy (3,500 r) to the pituitary gland five years earlier. He had mild diabetes mellitus, controlled with Orinase.

RESULTS

The results of the studies in the 8 children are presented in Table 2. HGH did not cause nitrogen retention in any of the 4 primordial dwarfs.

TABLE 2. EFFECT OF HGH IN DWARFISM

Patient	Wt. (Kg.)	HGH therapy		Calories per 24 hrs.	Nitrogen intake (Gm./24 hrs.)	Change in nitrogen balance (Gm./24 hrs.)	Change in urinary calcium* (mg./24 hrs.)
		Dose (mg./24 hrs.)	No. of days				
M.M.	15.3	5	6	1562	9.3	+0.1	+ 30
J.M.	8.7	5	6	845	4.6	+0.3	0
D.G.	35.2	5	6	1440	11.4	−0.4	+ 46
J.H.	13.0	10	6	1022	8.2	+0.2	+ 87
Car.K.	31.1	10	4	1806	11.3	+0.7	+148
Cat.K.	40.4	10	4	1925	11.4	+1.2	+ 64
J.W.	25.7	5	10	1968	14.0	−0.1	+ 32
		10	4	1968	14.0	0	+ 29
		5	8	1968	14.0	+0.8	− 16
		4	7	1968	14.0	+1.0	+ 18
P.B.	38.7	5	16	2012	11.7	−0.2	0

* These differences were calculated using the mean values for the appropriate balance periods.

Since the primordial dwarfs were resistant to the anabolic effects of HGH, other effects of HGH in this group are significant. In 3 of these patients, the urinary excretion of calcium increased. In Figure 1, the changes in urinary calcium and nitrogen balance are charted for one of the primordial dwarfs, for the normal girl, and for the girl with gonadal dysgenesis. The increase in urinary calcium was independent of the anabolic effect. Sodium retention occurred in 2 of the dwarfs, demonstrating that this phenomenon is also independent of nitrogen retention. In Figure 2, the effect of HGH on the plasma UFA level is compared with the effect of five hours' fasting alone, in both dwarfs and acromegalics. In

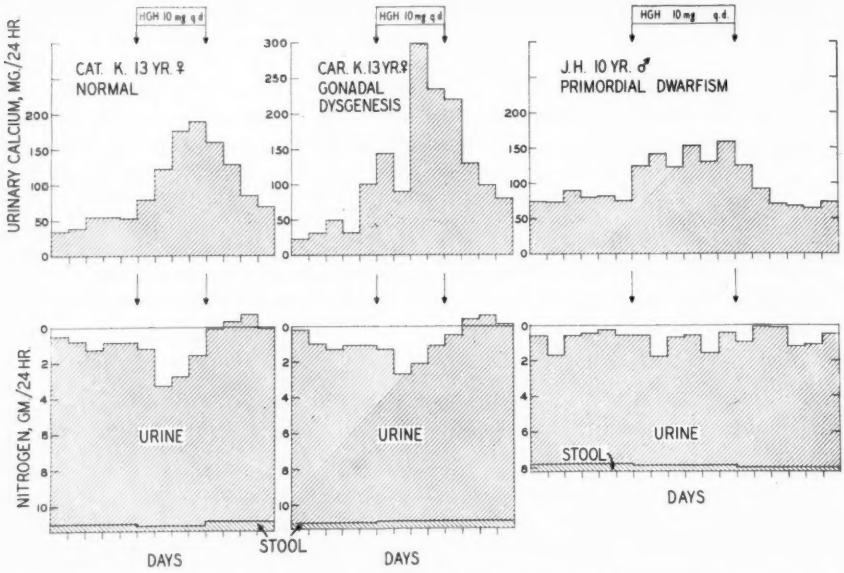

FIG. 1. Changes in urinary calcium and nitrogen balance in a normal girl, a girl with gonadal dysgenesis, and a boy with primordial dwarfism. The nitrogen intake is plotted from the zero line down. Superimposed on this are the values for urinary and fecal nitrogen. A positive balance is represented by a clear space below the zero line.

each instance the increase in plasma UFA concentration was significantly greater when HGH had been given. The zero-hour levels were 790 μEq. per liter on the control day and 910 μEq. per liter on the day of HGH administration. A similar UFA response to HGH has been noted in 3 normal children.

In contrast to the results in the primordial dwarfs was the nitrogen retention observed in 3 of the other 4 children. The nitrogen retained averaged 0.023, 0.030 and 0.027 Gm. per Kg. per twenty-four hours, or less than 1 Gm. daily. In the normal girl and the girl with gonadal dysgenesis (Fig. 1), there was a tendency for the anabolic effect to decrease even during these short periods of administration. The boy with achondroplasia (J.W., Table 2) had two courses of HGH, the first being ineffective and the second causing nitrogen retention. This is the only example of a patient failing to react to one preparation of HGH and responding to another. The changes in phosphorus and potassium balance were similar to those recorded in the previous paper. When nitrogen was retained, there was a slightly greater than theoretical retention of potassium and phosphorus. In the absence of nitrogen retention, the respective balances for these elements were unchanged.

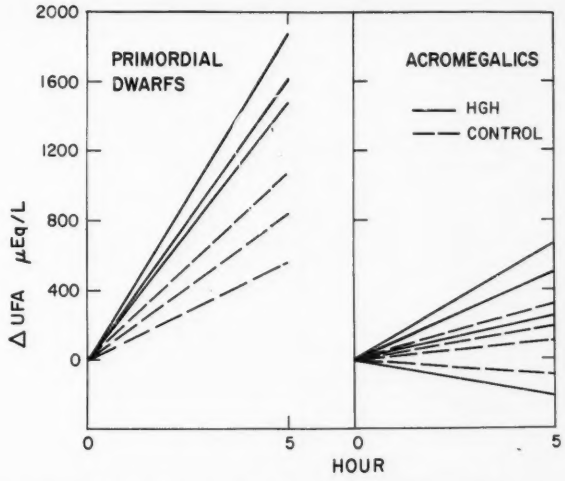

FIG. 2. Effect of HGH on the plasma UFA level in 3 children with primordial dwarfism and in 4 adult acromegalics. The controls are the plasma UFA levels after five hours of fasting plus injection of saline. The zero-hour levels were 790 μEq./L. on the control day and 910 μEq./L. on the day of HGH administration.

The blood sugar levels in Patient J.M. (dwarf) ranged between 35 and 50 mg. per 100 ml. after a 17-hour fast, and were not altered by HGH. Patient M.M. (dwarf) was not hypoglycemic after a 16-hour fast and HGH did not affect the blood sugar levels.

HGH did not promote nitrogen retention in any of the acromegalics during the initial studies (Table 3). In Patient J.D., there was a suggestion of an effect (Fig. 3) of HGH during the study six months following radiation therapy, but since we were unable to obtain a control period following HGH administration, the data were not conclusive. The other acromegalic who was studied before and after radiation therapy (M.M.) was refractory to HGH on both occasions. Neither of the male acromegalics responded to HGH with nitrogen retention in spite of the clinical impression and biochemical evidence of quiescent disease following radiotherapy. However, in the acromegalics, the hypercalciuric effect of HGH was observed in 5 or 6 studies (Table 3). Sodium retention did not occur.

The increase in plasma UFA concentration following administration of HGH was not as clear in the acromegalics as in the children (Fig. 2). There was considerable overlap between the change in UFA in response to fasting and the change in response to fasting plus HGH. Nevertheless, in 3 of the patients, the 5-hour UFA level was greater following HGH than following an injection of saline. More extensive testing will be necessary.

TABLE 3. EFFECT OF ANABOLIC AGENTS IN ACROMEGALY

Patient	Wt. (Kg.)	Drug	Dose (mg./24 hrs.)	Duration of therapy (days)	Calories per 24 hrs.	Nitrogen intake (Gm./24 hrs.)	Change in nitrogen balance* (Gm./24 hrs.)	Change in urinary calcium (mg./24 hrs.)
J.D. ♀	55.4	HGH	5	6	1789	9.4	−0.3	+ 47
	55.5	HGH	10	4	1789	9.4	−0.1	+ 51
	55.1	T.P.†	25	6	1789	9.4	+1.0	− 73
	56.7	HGH	5	6	1781	11.5	+1.0	+ 34
	56.7	HGH	10	3	1781	11.5	+1.3	+ 47
M.M. ♀	54.6	HGH	5	6	1879	8.8	+0.1	0
	54.8	HGH	10	4	1879	8.8	−0.4	0
	54.4	T.P.	25	4	1879	8.8	+1.9	− 70
	57.5	HGH	5	6	1470	9.3	0	+ 82
	57.7	HGH	10	4	1470	9.3	−0.3	+103
G.W. ♂	82.9	HGH	5	6	2556	12.1	+0.2	+129
		HGH	15	4	2556	12.1	−0.1	+144
		Fm.‡	10	4	2556	12.1	+2.1	− 81
O.W. ♂	70.3	HGH	5	6	2026	1.7	−0.2	+ 52

* The differences were calculated using the mean values for the appropriate balance periods.
† T.P. = Testosterone propionate.
‡ Fm. = Fluoxymesterone.

The administration of androgen resulted in nitrogen retention in each instance (Table 3). The nitrogen retained averaged 1.7 Gm. per day—roughly comparable to the degree of nitrogen retention observed in the normal adult under equivalent conditions.

The phosphorus and potassium balances as determined chemically agreed with the theoretical balances calculated from the changes in nitrogen and calcium excretion.

DISCUSSION

Raben (1) has shown that stimulation of linear growth can be produced in the panhypopituitary subject by HGH. Hutchings *et al.* (5) observed nitrogen retention and linear growth in a girl whose growth had become slowed following a high fever but who was otherwise normal. We are not aware of any reports dealing with the effect of HGH on primordial dwarfs.

It is apparent that the primordial dwarfs we studied were resistant to HGH. Although generalizations should not be attempted from such a small sample, one may conclude that an appreciable proportion of primordial dwarfs will not show nitrogen retention in response to HGH. These data imply that the inability to deposit nitrogen in response to growth hormone is closely associated with the failure of epiphyseal growth.

We have tested this hypothesis only once. Patient J.H. received 2.5

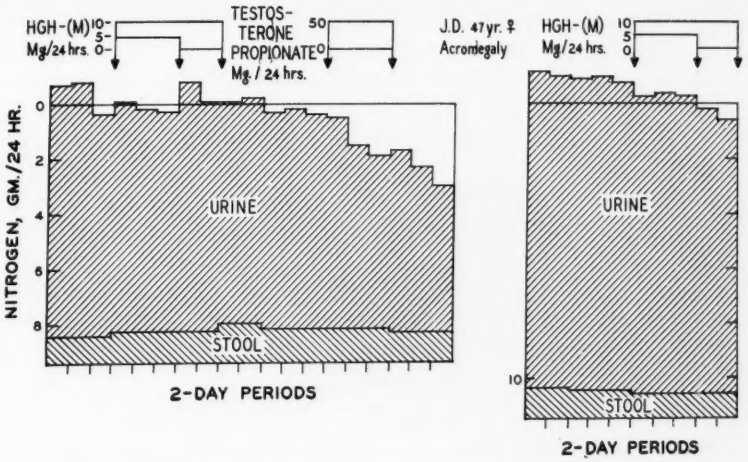

Fig. 3. Effect of HGH on nitrogen balance in a case of acromegaly. In contrast is the nitrogen retention during the period of testosterone therapy. Nitrogen intake is plotted as described in the legend of Figure 1.

mg. of HGH three times weekly for six months and no growth was observed. However, in another patient with panhypopituitarism due to craniopharyngioma, no growth occurred during four months of treatment with HGH. Henneman (6) failed to induce significant growth with HGH in 2 of 3 pituitary dwarfs. Thus the failure to induce linear growth in one primordial dwarf does not prove that HGH will not induce linear growth in any of these patients, since it has also failed in some dwarfs with panhypopituitarism.

HGH has almost uniformly induced in adults an increase in the level of plasma UFA (7, 8) greater than that associated with fasting. In the 3 primordial dwarfs tested, HGH had this effect. This increase in plasma UFA concentration and the presumed increased utilization of fat has been proposed as the mechanism for the protein-sparing and consequent nitrogen-retaining effect of HGH. However, although HGH caused a rise in the plasma UFA level in the primordial dwarf, there was no concomitant measurable retention of nitrogen. We would tentatively suggest that although the mobilization and utilization of fat are necessary for nitrogen retention, the ability of the tissues to retain nitrogen in response to HGH must further depend upon a direct effect of HGH on the responding tissue.

As further evidence that the primordial dwarf is not completely refractory to growth hormone, the increase in calcium excretion should be cited. The hypercalciuria and negative calcium balance of the acromegalic (9)

indicate that this effect is due to growth hormone *per se*, and not to some contaminating factor. Data are not available that would permit reasonable speculation about the mode of action of HGH with respect to hypercalciuria.

Nitrogen retention in 3 of the 4 children who were not primordial dwarfs was less than in adults, and there was a tendency toward escape from the effect of HGH. Grumbach (10) was unable to demonstrate growth following administration of HGH to premature infants, but Crigler *et al.* (11) reported significant nitrogen retention in an infant given 2 mg. of HGH daily. It is apparent that although in children HGH does not cause excessive nitrogen retention, as in the adult, the response to HGH differs only quantitatively from that of the adult, in sharp contrast to the complete lack of response in the primordial dwarf.

In the normal adult, HGH uniformly induces nitrogen retention (2). However, in 5 of the present studies in acromegalic subjects, there was no anabolic effect. In active acromegaly, the plasma levels of the sulfation factor (12) and of growth hormone (13, 14) are elevated; therefore the addition of exogenous growth hormone may not represent an appreciable increment. Three of our studies were performed in patients with apparently clinically quiescent disease, so this explanation would not appear to be applicable. The somewhat less plausible possibility is that a maximal tissue response to growth hormone had been obtained and that this was subsequently maintained in 3 of the 4 subjects. Definitive interpretation of these results must await correlative studies of the effects of exogenous HGH on plasma levels of endogenous HGH or sulfation factor.

The anabolic effect of the administered androgens demonstrated that the tissues were still capable of retaining nitrogen. Kochakian (15) found that the effects of growth hormone and testosterone are additive and Kinsell *et al.* (13) noted that testosterone promoted nitrogen retention in 2 acromegalics. These data imply different sites or mechanisms of deposition of protein that depend upon the nature of the anabolic agent.

In contrast to the discrepancies previously noted between the actual and theoretical retention of potassium and phosphorus following administration of HGH, there was close agreement in the testosterone studies. This is in accord with the suggestion that androgen therapy results in deposition of nitrogen at sites other than those acted upon by growth hormone.

HGH was not completely inactive in the acromegalics. In 5 of the 6 studies, urinary calcium excretion increased. There was also a suggestion of an increase in the plasma UFA level. These findings are so similar to those obtained in primordial dwarfism that they strengthen the hypothesis that the failure of the acromegalic to respond to HGH by nitrogen reten-

tion is due to an inability of the tissue to respond. This deficiency apparently persists after the pituitary adenoma has been adequately treated by x-ray therapy.

Acknowledgment

We are most grateful to Dr. Lawson Wilkins for the opportunity to study 3 of the primordial dwarfs. We thank Dr. C. H. Li, Dr. M. S. Raben and Dr. N. Brink for their preparations of human growth hormone. The devoted efforts of the nurses and dietitians of the Metabolic Ward made the studies of the children possible.

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THE ACTION OF 1,1,3-TRICYANO-2-AMINO-1-PROPENE (U-9189) ON THE THYROID GLAND OF THE RAT AND ITS EFFECTS IN HUMAN THYROTOXICOSIS*

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ABSTRACT

Studies of the antithyroid action of 1,1,3-tricyano-2-amino-1-propene (U-9189) were performed. In rats, this agent induces acute inhibition of the organic binding of iodine, suppresses the formation of thyroxine, and inhibits the conversion of monoiodotyrosine to diiodotyrosine. Following prolonged administration, these actions are supplemented by inhibition of the thyroidal iodide-concentrating mechanism. The antithyroid actions of U-9189 apparently also obtain in man, since this agent is capable of ameliorating human thyrotoxicosis. However, a high incidence of toxic reactions was observed in a small number of patients so treated.

DURING subacute toxicity studies of a compound thought to have possible value in the treatment of mental disorders, a dose-related hyperplasia of the thyroid gland was noted in rats and dogs (1). In view of the unusual chemical structure of this compound—1,1,3-tricyano-2-amino-1-propene or U-9189 and the dissimilarity between it and other goitrogenic agents, studies of the mechanism of its action on the thyroid gland were undertaken.

Prior observations conducted for as long as forty-two days in 20 human subjects had revealed no significant toxic manifestations save for occasional gastric distress, at dosage levels below 1.0 to 1.5 Gm. daily (1). Accordingly, a therapeutic trial of U-9189 was made in 6 patients with thyrotoxicosis.

The results indicate that, in rats, U-9189 is a potent goitrogen by virtue of its ability to inhibit diverse stages in the synthesis of thyroid hormone.

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In man, it can control thyrotoxicosis, as judged by clinical and laboratory criteria.

STUDIES IN ANIMALS

Methods and Results

Studies were performed in male rats of the Sprague-Dawley strain, each weighing 100 to 150 Gm. Unless otherwise indicated, animals were maintained with an unrestricted diet of Purina Chow and tap water. Injected drugs were administered intraperitoneally in 0.3 to 0.5 ml. of physiologic sodium chloride solution, except for propylthiouracil (PTU), which was dissolved in dilute alkali. Similar volumes of solvent were given to control animals. Both injection of material and sacrifice of animals in a single group were performed *ad seriatim*, so that uniform time relationships could be maintained. In experiments in which thyroid/plasma concentration ratios for inorganic iodide (T/P) were determined, animals were lightly anesthetized with ether. Blood was withdrawn from the abdominal aorta into heparinized syringes, and thyroidectomy was performed. T/P ratios were determined according to conventional techniques (2). Measurements of radioactivity were made in a well-type scintillation counter; when indicated, the radioactivity of a suitably diluted aliquot of the administered dose of carrier-free inorganic I^{131} was also determined. The nature and proportion of radioiodinated compounds in the thyroid glands were assessed by preparative, hydrolytic and analytic techniques described in detail elsewhere (3).

Experiment I. Six rats were given a single dose of U-9189 (10 mg. per Kg.), while 6 control animals received an injection of physiologic saline. Thirty minutes later, 0.5 μc. of inorganic I^{131} was administered. Four hours after receiving radioiodine, the animals were killed by a blow on the head. The thyroid glands were quickly removed and placed directly into counting tubes for assessment of their radioactivity.

U-9189 produced a highly significant ($P < .001$) decrease in the thyroidal uptake of I^{131}, from a control value of 2.9 ± 0.7 (mean $\pm$ standard deviation) to 0.4 ± 0.2 per cent of the administered dose.

Experiment II (*Fig. 1*). Eighteen rats were divided into three equal groups. One group received U-9189 in a dosage of 10 mg. per Kg., the second group was given 20 mg. per Kg., and the third (control) group received physiologic saline. Thirty minutes later, inorganic I^{131} was administered (4.0 μc. in control animals, 40.0 μc. in the others). Four hours thereafter, the thyroid glands were removed and counted individually for radioactivity. The pooled glands from each group were analyzed for their content of radioiodinated amino acids and inorganic I^{131} (3).

Percentile thyroidal uptakes of I^{131} were greatly decreased from control values (8.0 ± 4.3) by administration of either dose of U-9189 ($P < .01$). Uptakes were even lower in animals receiving 20 mg. per Kg. (0.6 ± 0.1) than in animals receiving the smaller dose (1.5 ± 1.2), but the difference between the two treatment groups was not statistically significant.

In the control group, inorganic I^{131} comprised only 5.4 per cent of the

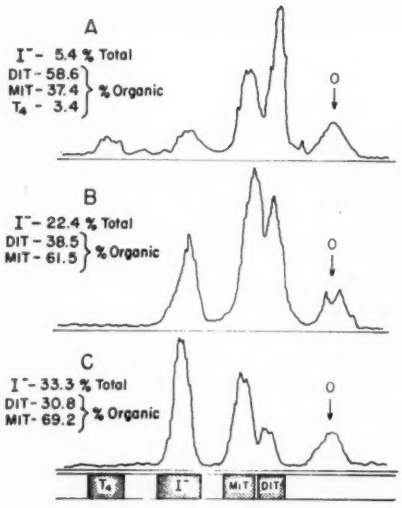

FIG. 1. The effect of U-9189 on the thyroidal synthesis of iodinated amino acids (Experiment II). Butanol-dioxane-ammonia chromatograms of pooled thyroid glands following tryptic hydrolysis. **A**, control groups; **B**, U-9189 (10 mg./Kg.); **C**, U-9189 (20 mg./Kg.). Animals killed four hours after I^{131} and four and a half hours after test drug.

total glandular radioiodine. Thyroxine*[1] contained 3.4 per cent, diodotyrosine* (DIT)* 58.6 per cent, and monoiodotyrosine* (MIT)* 37.9 per cent of the organically bound I^{131} (exclusive of unhydrolyzed origin material). Triiodothyronine* was not seen. Both doses of U-9189 increased the proportion of total glandular radioiodine present as inorganic iodide, abolished the synthesis of thyroxine* and decreased the DIT*/MIT* ratio. More pronounced changes were produced by the larger dose of the drug.

Experiment III (*Table 1*). Thyroid glands obtained from freshly killed sheep were transported to the laboratory on cracked ice. After being dissected free of fat and fascia, the glands were homogenized in 5 volumes of a modified KCl medium.[2] Solutions of methylmercaptoimidazole (MMI) or U-9189 in the homogenization medium, adjusted to pH 7.4, were added to the aliquots of homogenate to achieve for each drug a final concentration of 1×10^{-3}, 3×10^{-4} or 1×10^{-4} *M*. Final concentrations of homogenate represented a seven-fold dilution of the original thyroid tissue (W/V). Duplicate aliquots of control or drug-supplemented homogenates were pipetted into Warburg flasks. After equilibration in the respirometer at 38° C. under 100 per cent O_2, 1.0 μc. of inorganic I^{131} was tipped into each main compartment from the side-arm. One hour later, flasks were removed and the reaction was stopped by chilling and adding 1 drop of 1 per cent KI. A 20-μl. aliquot of homogenate from each vessel was pipetted into 1 ml. of a 1 per cent solution of human serum albumin. The resulting

[1] Asterisk indicates I^{131}-labeled compound.

[2] Homogenization medium: 10^{-3} *M* $MgSO_4$, 6×10^{-3} *M* phosphate buffer (pH 7.4), 0.14 *M* Kcl.

mixture was precipitated by the addition of 2 ml. of 10 per cent trichloracetic acid. Precipitates were washed three times with 5 per cent TCA. The radioactivity of both precipitates and pooled TCA-supernatants was determined. The percentile organification of I^{131} was calculated as the ratio of TCA-precipitable to total I^{131}.

TABLE 1. THE EFFECT OF VARYING CONCENTRATIONS OF METHYLMERCAPTOIMIDAZOLE (TAPAZOLE) AND U-9189 ON ORGANIC IODINATIONS IN A HOMOGENATE OF SHEEP THYROID GLAND

Concentration of antithyroid agent	Organic iodinations (% added I^{131}-iodide)	
	Tapazole	U-9189
0	11.60	11.60
1×10^{-4} *M*	0.21	0.29
3×10^{-4} *M*	0.19	0.21
1×10^{-3} *M*	0.17	0.18

Values obtained in duplicate vessels did not differ from one another by more than 12 per cent and were therefore averaged. MMI and U-9189 both produced a pronounced inhibition of organic iodinations. At the highest concentration, no difference between the two drugs could be discerned. At the lowest concentration, organic iodinations in vessels containing U-9189, although only one-fortieth of control values, were 40 per cent greater than in vessels containing MMI.

Experiment IV (*Table 2, Fig. 2*). Thirty-six rats were divided into six equal groups. Of these, three groups were maintained with a Remington

TABLE 2. THE EFFECT OF VARYING DIETARY IODINE ON THE GOITROGENIC RESPONSE TO PROPYLTHIOURACIL AND TO U-9189

No. of animals	Diet additive*	Thyroid weight (mg./100 Gm. body wt.)	Thyroidal I^{131} uptake		Inorganic I^{131} (% thyroid I^{131})
			(% dose)	(% dose/100 mg. thyroid)	
		Remington diet			
6	0	15.5±2.4	—	—	—
6	PTU (0.1%)	33.8±6.2**	—	—	—
6	U-9189 (0.1%)	33.2±5.2**	—	—	—
		Remington diet+KI (750 μg./100 Gm.)			
6	0	10.7±2.2	1.4±0.9	7.8±4.0	4.6
6	PTU (0.1%)	29.8±2.7**	3.3±0.5**	6.3±1.2	98.0
6	U-9189 (0.1%)	27.7±9.2**	1.5±0.8†	3.3±1.7**†	81.8

* PTU (propylthiouracil), or U-9189 (1,1,3-tricyano-2-amino-1-propene) added to basic diet for one week.

** Significantly different from control value ($P < .01$).

† Significantly different from value in PTU-treated group ($P < .02$).

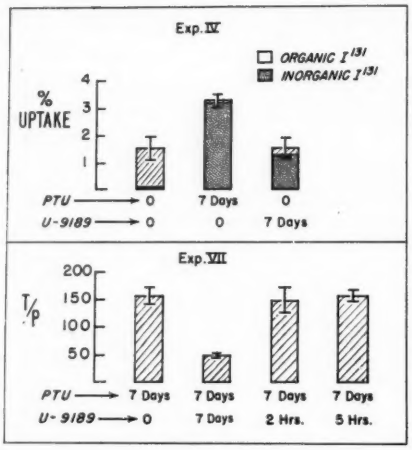

FIG. 2. The effect of U-9189 and propylthiouracil on iodine metabolism in the rat thyroid (Experiments IV and VII). PTU (propylthiouracil) and U-9189 administered in a concentration of 0.1% in the diet, except for the last two groups in Experiment VII, in which U-9189 (20 mg./Kg.) was administered intraperitoneally.

low-iodine diet,[3] while the remaining three groups were fed the same diet to which KI in the amount of 750 μg. per 100 Gm. had been added. Within these major dietary categories, the feed of one group was supplemented with 0.1 per cent propylthiouracil (PTU), that of another with 0.1 per cent U-9189, while the third group served as either a low-iodine or a high-iodine control. After one week, an injection of inorganic I^{131} was given. Three hours later, each animal was weighed and killed. The thyroids were dissected free and weighed on a torsion balance. Glands were counted for radioactivity individually; those from each group were pooled, homogenized, and chromatographed without prior hydrolysis. The proportions of I^{131} present in the homogenate in both inorganic and organically-bound (chromatographically-immobile) forms were determined.

The goitrogenic effect of the Remington diet was evident in the moderate increase in thyroidal mass in animals given this low-iodine diet. Goiters resulting from the administration of U-9189 were as large as those induced by PTU and were not significantly decreased in size by supplementation of the Remington diet with potassium iodide.

Radioiodine studies were performed only in animals which had received the high-iodine diet. Total thyroidal uptakes of I^{131} in animals given PTU were significantly greater than control values ($P < .001$), whereas those in the U-9189-treated group were approximately normal. However, when uptakes were calculated on the basis of glandular mass, values in PTU-treated and control groups were essentially the same, whereas those in animals given U-9189 were significantly decreased ($P < .01$). Inorganic iodide comprised only 4.6 per cent of the I^{131} present in the pooled thyroid glands of the control animals. In contrast, radioiodide accounted for vir-

[3] Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

tually all of the I^{131} in PTU-treated rats and for 82 per cent of the I^{131} in U-9189-treated rats.

Thus, as compared to control values, the percentage of administered I^{131} incorporated into organic components was greatly reduced in animals given U-9189, while glandular radioiodide was greatly increased. On the other hand, in animals given U-9189, accumulation of inorganic radioiodine was far less than in animals given PTU.

Experiment V. Eighteen rats were divided into three equal groups, treated in the following manner:

0 time: PTU, 5.0 mg. intraperitoneally.
30 minutes: 0.5 ml. of physiologic saline (Group A); U-9189, 10 mg. per Kg. (Group B); U-9189, 20 mg. per Kg. (Group C).
90 minutes: 2.0 μc. of I^{131}.
150 minutes: T/P ratios determined.

Mean T/P ratios in Groups A, B, and C were 26 ± 5.1, 18.1 ± 3.7, and 19.3 ± 4.0, respectively. Thus, U-9189 had no effect on the iodide-concentrating capacity of rat thyroid gland during acute PTU blockade.

Experiment VI. The exact protocol of Experiment V was repeated. T/P ratios averaged 11.8 ± 4.4 in control animals and 9.2 ± 2.4 in animals given the smaller dose of U-9189. The larger dose of U-9189 produced a significant ($P < .05$) reduction of the T/P ratio to 6.4 ± 1.4.

Experiment VII (*Fig. 2*). Eighteen rats were maintained with ground Purina Chow containing 0.1 per cent PTU. An additional 6 rats received the same ration, supplemented with both PTU (0.1 per cent) and U-9189 (0.1 per cent). After one week, rats receiving only PTU were divided into three groups of 6 animals each. Of these, the first group received U-9189 in doses of 20 mg. per Kg. ninety minutes before sacrifice, and the second group received a similar dose five hours beforehand. Animals in the third group served as PTU-treated controls. All animals received inorganic I^{131} sixty minutes before sacrifice.

The goitrogenic effect of dietary PTU was not significantly altered by addition of an equal concentration of U-9189. Animals given PTU alone displayed very high T/P ratios for inorganic iodide. These were unaffected by U-9189 in doses of 20 mg. per Kg. acting for ninety minutes or for five hours. However, T/P ratios were reduced by about 70 per cent ($P < .001$) in animals receiving in their diet for one week, U-9189 in addition to PTU.

STUDIES IN HUMAN SUBJECTS

A trial of therapy with U-9189 was carried out in 6 patients with thyrotoxicosis. The clinical features are presented in Table 3. The initial dosage was 100 mg. every eight hours orally, but this was usually increased as

the patient's tolerance and response were assessed. Partial or complete alleviation of thyrotoxicosis was observed in all patients in whom therapy was continued for at least a month. Figure 3 shows the effect of U-9189 on the clinical symptoms, pulse rate, serum protein-bound iodine level, and basal metabolic rate in a case of diffuse toxic goiter (Patient 1, Table 3). Control was maintained during prolonged treatment for slightly over four months. There was no evidence of drug toxicity. When U-9189 was discontinued, symptoms returned and the PBI level and BMR rose. The response of Patient 6 was notable in that she had failed to improve while receiving PTU or MMI in dosages of 1200 mg. and 90 mg. daily,

TABLE 3. RESPONSE TO U-9189 IN PATIENTS WITH THYROTOXICOSIS

Patient	Age & sex	Diagnosis	Response to therapy	Maintenance dosage of U-9189 (mg./day)	Duration of therapy	Comment
1	21 M	Diffuse toxic goiter	Remission of thyrotoxicosis	400	4 mos.	—
2	65 F	Toxic nodular goiter; incomplete response to I^{131}	Remission of thyrotoxicosis	450	4 mos.	Therapy discont'd because of paresthesias in hands and feet.
3	34 F	Diffuse toxic goiter; recurrence after I^{131}	Remission of thyrotoxicosis	600	3½ mos.	Therapy discont'd because of paresthesias in hands and feet.
4	22 F	Diffuse toxic goiter; recurrence after PTU therapy	—	300	7 days	Therapy discont'd because of fever and urticarial rash.
5	24 F	Diffuse toxic goiter	Remission of thyrotoxicosis	600	1 mo.	Therapy discont'd because of urticarial rash
6	22 F	Diffuse toxic goiter; moderate infiltrative ophthalmopathy; severe thyrotoxicosis resistant to PTU and Tapazole	Partial remission of thyrotoxicosis	1500	3 mos.	Ophthalmopathy unaffected.

respectively. With 1500 mg. of U-9189 daily, a partial response was obtained, but thyrotoxicity was not completely controlled.

Toxic reactions were evident in 4 of the 6 subjects. In Patients 4 and 5, an urticarial rash followed seven days and one month of treatment, respectively. In Patients 2 and 3 paresthesias of the fingers and toes developed after several months of treatment. Clinical and laboratory observations indicated this was not associated with therapeutic myxedema. Objective sensory and motor changes could not be detected. Since these patients were being treated concurrently and the paresthesias developed at almost the same time, these symptoms had evidently been produced by the drug. Therapy was therefore withdrawn. In both patients, sensory symptoms subsided within two weeks after discontinuance of treatment.

Aside from occasional gastric distress, other toxic manifestations were not noted. Hematologic changes were confined to alterations in the differential leukocyte count incident to relief of thyrotoxicosis. Periodic urine examinations revealed no abnormality, save for the appearance of an

orange-red pigment in the urine of the 2 patients who received the largest doses. This pigment, observed in animals and also during earlier studies of toxicity in man, apparently is not blood, blood pigment or bile pigment (1).

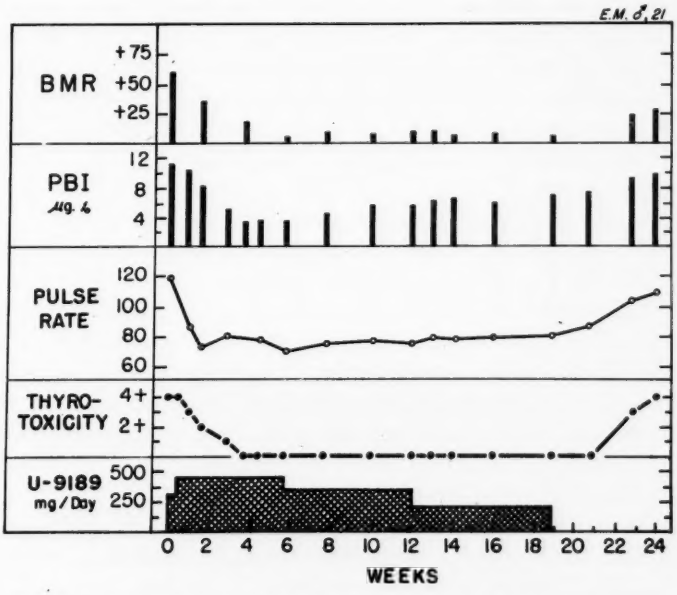

FIG. 3. The response to U-9189 in a patient with diffuse toxic goiter.

DISCUSSION

From results of the present and other studies, U-9189 appears to be an agent with diverse actions in the thyroid gland and elsewhere. Thus, it has been reported to have a beneficial effect in certain psychopathologic conditions (4) and to induce increases in the concentration of nucleoprotein, succinoxidase and cytochrome oxidase in certain cells in the central nervous system (5). More recently it has been reported that U-9189 uncouples oxidative phosphorylation in rabbit liver mitochondria during oxidation of alpha-ketoglutarate (6). In the present experiments, U-9189 proved to be a potent antithyroid agent with actions upon the synthesis of thyroid hormone that are multiple and complex.

The first several experiments were concerned with elucidating the effects of U-9189 on the organic binding of iodine and the synthesis of iodinated amino acids. In Experiments I and II, this agent acutely reduced the thyroidal accumulation of I^{131}. In the latter experiment, a pronounced, dose-

related increase in the proportion of thyroidal radioiodine present as iodide indicated that this agent, like those of the thiourylene series (7), is capable of blocking the organification of thyroidal iodide. Concomitantly, it appeared to inhibit the coupling of iodotyrosines to form iodothyronines, and to retard the formation of DIT from MIT. In these respects, the actions of U-9189 appeared similar to those recently described for PTU (3, 8) and for high doses of inorganic iodine (9) during induction of the Wolff-Chaikoff effect (10). Additional evidence of the ability of U-9189 to inhibit organic-binding of iodine was obtained in experiments performed with thyroid homogenates, in which the inhibitory potency of this agent compared favorably with that of MMI.

The fourth experiment, however, provided evidence of a difference in the actions of U-9189 and the thiourylenes. Here, in animals treated with propylthiouracil, the classic inhibition of organic binding was evident in the virtual absence of bound I^{131} in direct chromatograms of thyroid homogenates. In animals receiving U-9189, inhibition of organic binding was not complete, since a small percentage of glandular I^{131} was found in organic fractions. Despite this, the total uptake of I^{131} was considerably less than that in PTU-treated animals with thyroid glands of comparable weight. This finding suggested that U-9189 might also have inhibited the thyroidal mechanism for concentrating inorganic iodide.

Nevertheless, an alternative explanation required consideration. Since the inhibition of organic binding was less nearly complete with U-9189 than with PTU, there may have been some formation of active hormone in animals given the former drug. As a result, thyrotropic stimulation of the iodide-concentrating mechanism may have been less intense. This possibility was rendered unlikely by the observation that the goitrogenic effects of U-9189 were as great as those of PTU. In view of the results obtained in Experiment II, it seemed probable that the organically bound iodine formed in animals given U-9189 was comprised mainly of iodotyrosines and contained few, if any, hormonally active moieties. If this were so, and U-9189 did indeed inhibit the iodide-concentrating mechanism, then failure of supplementary iodine to decrease significantly the size of goiters formed in animals given U-9189 and a low-iodine diet could be explained by the concomitant inhibition of iodothyronine synthesis induced by this agent.

In the light of these considerations, experiments were performed to assess directly the effect of U-9189 upon the iodide-concentrating mechanism. Experiments V and VI were disappointing in that they indicated that the acute effects of U-9189 on this function were inconstant and slight. The explanation of earlier results (Experiment IV) was therefore left in doubt. Two possibilities were apparent. First, the inhibitory effects

of U-9189 on the iodide-concentrating mechanism might require prolonged administration of the drug. Second, the inhibition might be effected only in thyroid glands in which the iodide-concentrating mechanism was intensely stimulated by thyrotropin. Accordingly, Experiment VII was undertaken. Here, the effects of both chronically and acutely administered U-9189 were assessed in animals chronically treated with PTU. The results indicated that U-9189 is indeed capable of inhibiting the iodide-concentrating mechanism. This effect is more closely related to the duration of administration than to the activity of the mechanism itself.

Thus, U-9189 would appear to inhibit all stages of iodine metabolism concerned with the synthesis of active thyroid hormone. The present studies have not attempted to learn whether this agent also influences other aspects of thyroid hormone metabolism, such as hydrolysis of thyroglobulin, dehalogenation of iodotyrosines, or, as increasing evidence indicates is the case with PTU, the extrathyroidal disposal of hormone (11, 12).

The mechanism of action of U-9189 in relation to its chemical structure remains obscure. The structural formula suggests the possibility that its action may result from the intrathyroidal accumulation of cyanide. In thyroid slices, cyanide is a potent inhibitor of iodide trapping (13, 14) and it has also been found to decrease organic binding of I^{131} in thyroid homogenates (15, 16). On the basis of such *in vitro* studies, it seems unlikely that quantities of cyanide insufficient to inhibit the concentration of iodide would have profoundly decreased organic binding.

A second possible product of the metabolism of U-9189 might be thiocyanate. This, too, is a potent inhibitor of the iodide-concentrating mechanism (2) and also retards organic binding of iodine in thyroid homogenates (16). However, it seems unlikely that formation of thiocyanate can alone account for the effects of U-9189. First, doses of thiocyanate which reduce the total thyroidal uptake of I^{131} also decrease T/P iodide ratios. These effects were dissociated in the case of U-9189. Second, doses of thiocyanate which virtually abolish the thyroidal uptake of I^{131} do not alter the pattern of radioiodinated amino acids within the thyroid gland (17). In contrast, reduction of I^{131} uptake by U-9189 is associated with qualitative changes in hormonal synthesis more nearly resembling those induced by PTU. Finally, the goitrogenic action of thiocyanate is decreased or abolished by increasing dietary iodine (18), whereas the goitrogenicity of U-9189 was affected little if at all. Thus, although it remains possible that the delayed inhibition of the iodide-concentrating mechanism produced by U-9189 is due to progressive accumulation of thiocyanate, it seems unlikely that the acute effects of U-9189 on organic binding can be explained on this basis.

The observation that U-9189, like dinitrophenol, is capable of

uncoupling oxidative phosphorylation *in vitro* suggests another possible mechanism of action within the thyroid gland. The iodide-concentrating mechanism has been shown to be highly sensitive to the action of dinitrophenol and is presumably dependent on continued generation of phosphate-bond energy (13). In contrast, organic iodinations in thyroid homogenates are little if at all influenced by dinitrophenol (15, 19). Since the acute inhibitory effects of U-9189 are preponderantly exerted on the organic-binding mechanism rather than on the iodide-concentrating mechanism, it seems unlikely that uncoupling of oxidative phosphorylation within the thyroid gland is the sole action of this agent. Indeed, the extent to which U-9189 inhibits oxidative phosphorylations *in vivo* is uncertain. Dinitrophenol inhibits the formation of goiters in rats given PTU (20). As shown in Experiment VII, U-9189 had no such action.

The antithyroid actions of U-9189 are also produced in humans and can be employed to alleviate the thyrotoxicosis of Graves' disease or of toxic nodular goiter. Unfortunately, however, there was a high incidence of toxic reactions in this small group of patients; 2 of the 6 displayed cutaneous hypersensitivity and in 2 others neurologic symptoms developed. This would suggest that U-9189 *per se* has little place in the clinical armamentarium. Nevertheless, it has a seemingly unique ability to inhibit both iodide-concentrating and organic-binding mechanisms *in vivo*. Alterations in molecular structure to render it less toxic while retaining its unusual antithyroid action might provide an agent of considerable therapeutic value.

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DETERMINATION OF ADRENOCORTICOTROPIN IN HUMAN URINE BY A BENZOIC-ACID ADSORPTION METHOD

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ABSTRACT

Benzoic-acid adsorption was successfully used for the extraction of adrenocorticotropin (ACTH) from human urine to be assayed by the ascorbic-acid depletion (AAD) method in hypophysectomized rats. In 25 normal subjects the AAD induced by 26 such extracts of 24-hour urine was 51 ± 6.5 mg. per 100 Gm. of adrenal (mean $\pm$S.E.), whereas the value for 10 extracts of 12-hour urine from 4 patients with Addison's disease was 136 ± 14.3 mg. ($P < 0.001$). The ACTH content of a normal 24-hour specimen of urine was therefore estimated to be approximately 0.25–0.5 milliunit, whereas in Addison's disease the value may reach 1–2 milliunits per twelve hours. In 7 patients with hypopituitarism (12 extracts of 24-hour urine) and 12 patients receiving steroid therapy (18 extracts of 24-hour urine), significant decreases in ACTH were noted (23 ± 8.7 mg., $P < 0.02$; and 15 ± 8.0 mg., $P < 0.01$, respectively). In 5 cases of anorexia nervosa (5 extracts) there was no significant difference, either from normal subjects or from patients with hypopituitarism. Among 8 cases of Cushing's syndrome (14 extracts), 2 of 6 cases with bilateral adrenal hyperplasia showed high AAD values, whereas both cases with unilateral adrenal adenoma showed low AAD values.

INTRODUCTION

ALTHOUGH evidence has been accumulating on the appearance of adrenocorticotropin (ACTH) in human urine, reports on its quantitative estimation are conflicting. Locke *et al.* (1) failed to find ACTH in normal urine and reviewed the literature. Rubin *et al.* (2), on the other hand, found more than 1 U.S.P. unit per liter of fresh urine. One of us (3) reported a level of approximately 0.25 milliunit per twenty-four hours, using oxycellulose adsorption and assay by ascorbic-acid depletion. This method was further used by Nelson *et al.* (4), Tasaka *et al.* (5), and Katsuki (6), with similar results. In our laboratory, however, the oxycellulose tech-

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nic was found to yield inconsistent recovery values when applied to urine samples. The use of benzoic acid for the extraction of ACTH from urine was therefore studied, employing the technic of Takahashi and Lerner (7) for the extraction of melanophore-stimulating hormone (MSH) from urine.

METHOD AND MATERIAL

Method

Urine was collected in 24-hour samples at room temperature with 30 ml. of 6*N* HCl as a preservative. Immediately after the end of the collection, the samples were brought to pH 5.5 with 40 per cent NaOH and 6*N* HCl using an Iio glass electrode pH meter. Ethanol saturated with benzoic acid (5 per cent of the urine volume) was rapidly injected into the sample through a 50-ml. syringe. After the mixture was thoroughly stirred for one hour, the precipitate was collected by filtering the mixture through Whatman No. 20 filter paper on a Buchner funnel, washed three times (100 ml. each time) with distilled water saturated with benzoic acid, and dried in an oven at 70°C. for six hours. The dried cake of benzoic acid was mechanically broken and dissolved four times in 100 ml. of ether, previously washed with $\frac{1}{5}$ volume of 10 per cent ferrous sulfate. The combined mixture was centrifuged at 3000 r.p.m. for fifteen minutes, following the addition of 2 ml. of 0.005*N* HCl. The aqueous layer was transferred to a test tube, heated in a boiling-water bath for five minutes, and centrifuged at 2000 r.p.m. for five minutes to separate the supernatant fluid from the precipitate. The supernatant containing the active fraction was preserved in a refrigerator for assay. Adrenal ascorbic-acid depletion in hypophysectomized rats (8) was the method used for the assay of ACTH. Recovery experiments were performed after dissolving a known amount of U.S.P. corticotropin reference standard in alkali-preserved urine. In each urine sample, 17-hydroxycorticosteroids (17-OH-CS) and 17-ketosteroids (17-KS) were also determined by the methods of Glenn and Nelson (9) and Drekter *et al.* (10), respectively.

Subjects

The studies were carried out on 25 normal subjects, 4 cases of Addison's disease, 7 cases of hypopituitarism, 12 patients receiving adrenocortical steroid therapy, 5 cases of anorexia nervosa, 7 cases of Cushing's syndrome, and 11 patients with miscellaneous endocrine disorders.

RESULTS

As shown in Table 1, the recovery rate calculated by the method of Pugsley (11) appeared to be satisfactory in a simultaneous assay.

In 26 extracts of 24-hour urine from 25 normal subjects (Table 2) the mean ascorbic acid depletion (AAD) was 51 ± 6.5 mg. per 100 Gm. of adrenal weight—significantly higher than the depletion induced by physiologic saline ($P < 0.001$). This corresponds to approximately 0.25–0.5 milliunit of ACTH.

In all of the 4 cases of Addison's disease (Table 3) 10 extracts of 12-hour urine yielded ACTH values that were definitely higher than normal, the mean being 136 ± 14.3 ($P < 0.001$). In 2 of the 4 cases these values decreased after replacement therapy.

TABLE 1. STANDARD CURVE AND RECOVERY EXPERIMENTS

	ACTH (m. unit)		Rat No.	Ascorbic-acid depletion (mg./100 Gm. adrenal)	Mean ±S.E.	*b*	*s*	λ
USP standard ACTH	0		7	−19, 13, 21, −10, −7, 0, −1	0 ± 5.2	106	30	0.28
	0.25		6	38, 90, 16, 58, 25, 36	44 ± 10.9			
	0.5		9	90, 85, 77, 61, 30, 41, 60, 43, 109	66 ± 8.6			
	1.0		6	118, 88, 112, 91, 68, 84	94 ± 7.6			
	2.0		13	106, 128, 145, 162, 143, 93, 105, 104, 132, 227, 108, 150, 169	136 ± 10.1			
Recovered ACTH	A	0.5	5	24, 51, 61, 75, 81	58 ± 10.1	105	33	0.31
		2.0	5	87, 102, 112, 193, 114	122 ± 18.5			
	B	0.5	4	87, 43, 67, 84	70 ± 10.9	65	29	0.44
		2.0	5	101, 162, 81, 122, 79	109 ± 15.4			

Recovery rate (5% fiducial limit): A, 77.8% (96.0–63.1)
B, 75.1% (94.0–60.0).
(Recovery A was valid by χ^2 test at 95%, but recovery B was not.)

TABLE 2. ACTH AND STEROIDS IN URINE OF NORMAL SUBJECTS

No.	Subject	Age & sex	ACTH (AAD, mg./100 Gm.)	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)
1	F.I.	15 M	30	1.9	6.5
2	T.I.	16 M	57	2.7	10.4
3	Y.O.	19 M	63	2.9	7.8
4	S.O.	20 M	80	2.8	8.5
5	N.I.	21 M	72	3.2	14.0
6	F.S.	21 M	70	2.1	7.3
7	Y.Y.	22 F	78	4.3	6.5
8	K.T.	22 F	−27	2.7	7.3
9	H.I.	25 F	67	2.0	7.0
10	H.N.	25 M	73	4.5	9.0
11	A.N.	27 F	9	4.5	11.0
12	K.O.	28 M	52	1.8	9.2
13	S.T.	29 F	82	2.0	4.3
14	Y.O.	30 F	45	4.0	7.0
15	T.I.	31 F	38	3.2	9.8
16	S.H.	31 M	51	2.4	7.4
17	Y.K.	34 F	63	2.3	9.0
18	H.K.	35 M	70	3.0	7.6
19	S.T.	35 F	58	3.5	8.8
20	T.Y.	36 M	94	2.6	12.4
21	S.F.	45 F	6	2.5	10.8
22	K.N.	46 M	55	1.8	8.6
23			7	2.1	8.4
24	Y.N.	49 M	−29	3.0	15.0
25	S.S.	54 F	69	2.3	5.0
26	T.T.	70 M	90	2.5	12.3
Mean ±S.E.			*51* ±*6.5*	*2.8* ±*0.16*	*8.9* ±*0.50*

TABLE 3. ACTH AND STEROIDS IN URINE OF PATIENTS WITH ADDISON'S DISEASE AND HYPOPITUITARISM

Addison's disease (12-hour urine)

	Patient No.	Name	Age & sex	ACTH (AAD, mg./100 Gm.)	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)
Before treatment	1	N.F.	35 F	105	0.4	4.5
	2	S.H.	37 M	236 190	0.3	0.5
	3	K.N.	39 M	93 123	0.3	5.2
	4	H.K.	42 M	150 107 127 103 131	0.2 0.3	4.3 5.7
Mean ± S.E.				*136 ± 14.3*	*0.3 ± 0.03*	*4.0 ± 0.92*
After treatment	3	K.N.	39 M	60 (1 wk.) −90 (1 mo.)		
	4	H.K.	42 M	−30 (3 wks.)		
Mean ± S.E.				*−20 ± 43*		

Hypopituitarism

Patient No.	Name	Age & sex	Diagnosis	ACTH (AAD, mg./100 Gm.)	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)
1	K.T.	9 F	Pituitary dwarfism	1 49 13 7	0.6 1.6 0.4	1.3 1.1 1.3
2	Y.K.	29 F	Simmonds'disease	33 23 22	0 0.8 1.4	3.0 4.1 2.2
3	T.I.	32 F	Sheehan's disease	−16	0.8	2.8
4	J.U.	34 F	Sheehan's disease	14	0.4	2.0
5	K.T.	36 M	Simmonds' disease	31	0.7	5.4
6	K.M.	37 F	Sheehan's disease	100	0.5	3.4
7	T.I.	39 M	Simmonds' disease	−4	0.4	3.7
Mean ± S.E.				*23 ± 8.7*	*0.6 ± 0.14*	*2.7 ± 0.40*

Assay of 12 extracts of 24-hour urine from 7 cases of hypopituitarism (Table 3) indicated a decreased amount of ACTH as shown by a mean AAD value of 23 ± 8.7 ($P < 0.02$).

In 12 patients receiving synthetic adrenocortical steroids (Table 4), 18 extracts of 24-urine yielded a mean AAD value of 15 ± 8.0—significantly lower than in the normal subjects ($P < 0.01$).

TABLE 4. ACTH AND STEROIDS IN URINE OF PATIENTS TREATED WITH SYNTHETIC CORTICOSTEROIDS

No.	Patient	Age & sex	Diagnosis	Steroid therapy*		ACTH (AAD, mg./100 Gm.)	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)
				Tot. dose (mg.)	Duration (days)			
1	F.I.	15 F	Pulseless disease	D 56	14	− 5	0	0.5
2	M.S.	19 F	Pulm. tuberculosis	P140	7	−21	1.0	4.4
3	F.S.	21 M	Pulm. tuberculosis	P280	14	25	0.8	3.7
4	H.O.	25 M	Pleurisy	P140	7	32	0.3	5.6
5	A.N.	25 M	Pleurisy	P 21	7	40	0.9	3.5
6	T.I.	26 M	Hemophilia	M112	7	−35	1.5	3.2
7	K.O.	31 F	Lupus erythematodes	M336	21	36	2.8	3.2
8				D 28	7	− 4	1.1	3.5
9				D 56	14	30	1.2	3.0
10				D 84	21	80	2.9	3.5
11				D112	28	57	1.8	0.5
12				D140	35	66	1.5	0.9
13	K.O.	32 M	Spinal arachnoiditis	D700	35	−12	0.5	6.2
14	M.M.	35 F	Spinal cord tumor	D 63	21	15	0.8	2.2
15	S.Y.	59 F	Spinal cord tumor	D 21	7	−26	1.0	7.6
16				D 42	14	3	1.2	4.6
17	S.T.	42 M	Polyneuritis	D 84†	28	−26	0.4	1.8
18	T.T.	70 M	Acute hepatitis	D118‡	54	18	1.7	6.8
			Mean ± S.E.			*15*±*8.0*	*1.1*±*0.18*	*3.6*±*0.48*

* D = Dexamethasone; P = Prednisolone; M = Methylprednisolone.
† Thirty days after discontinuation.
‡ Fourteen days after discontinuation.

Five cases of anorexia nervosa (Table 5) demonstrated urinary ACTH excretion not significantly different from normal. In all of these patients, urinary 17-OH-CS and 17-KS values were significantly below normal.

Among 8 patients with Cushing's syndrome (Table 6), including 6 with adrenal hyperplasia and 2 with adenoma, urinary ACTH values were increased only in 2 of the cases of hyperplasia.

DISCUSSION

In view of the fact that determination of ACTH in blood requires a huge amount of peripheral blood—as much as 200 ml. in normal subjects (3)—its clinical application is impractical. Although the physiologic and pathologic significance of ACTH in urine has not been completely clarified, it might furnish a useful index of ACTH secretion for clinical purposes. The definite increase of urinary ACTH in Addison's disease strongly suggests the parallelism between ACTH in blood and urine, in view of the already recognized increase of blood ACTH in this condition (12).

For the diagnosis of hypopituitarism, however, urinary ACTH would provide a less decisive index, due to the limited sensitivity of the assay. The significantly lower values obtained in the patients being treated with synthetic corticosteroids might be considered as direct evidence of decreased ACTH secretion in such cases of iatrogenic adrenocortical insufficiency.

TABLE 5. ACTH AND STEROIDS IN URINE OF PATIENTS WITH MISCELLANEOUS CONDITIONS

No.	Name	Age & sex	Diagnosis	ACTH (AAD, mg./100 Gm.)	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)
1	Y.S.	15 F	Anorexia nervosa	54	0.7	3.2
2	A.N.	16 F	Anorexia nervosa	25	1.5	5.0
3	S.O.	21 F	Anorexia nervosa	37	1.0	6.5
4	S.Y.	27 F	Anorexia nervosa	107	0.9	4.8
5	K.T.	28 F	Anorexia nervosa	−20	2.8	5.8
Mean ± S.E.				*40 ± 20.6*	*1.4 ± 0.38*	*5.0 ± 0.55*
6	Y.S.	25 M	Acromegaly	30	4.0	11.9
7	M.H.	30 M	Acromegaly	77 70	0.8	5.1
8	H.I.	32 F	Acromegaly + diabetes mellitus	113	2.1	13.7
9	Y.M.	29 M	Hyperthyroidism	− 9 48 18	4.7	9.0
10	Y.N.	26 M	Myxedema treated treated	1 70 63	1.7 2.4	5.7 5.4
11	S.M.	48 M	Myxedema treated	18 70	1.8 3.6	7.7 14.1
12	Y.U.	17 F	Turner's syndrome	− 4	0.8	4.5
13	T.F.	11 F	Precocity	75	3.2	7.0
14	T.S.	9 M	Obesity	−27	2.0	4.3
15	N.K.	13 M	Obesity	−19	1.7	9.2
16 17	H.S.	8 F	Obesity	45 −32	5.0	5.1

The problem is more complicated in Cushing's syndrome. Our data at least indicate that there may be an increased amount of ACTH in the urine in some patients with this syndrome. Sydnor and Sayers (13), Sprague (14), and Paris *et al.* (15), failed to find an increased amount of ACTH in the blood of patients with Cushing's syndrome. Recently, however, Nelson, Meakin and Thorn (16) reported high plasma ACTH levels in patients in whom pituitary tumors had developed following adrenalectomy for Cushing's syndrome. Whether such variable results are related to the pathologic nature of the disease, chronologic elements, or other factors, awaits further study in association with the elucidation of the role of pituitary in the pathogenesis of this syndrome. Whenever the functional status of the pituitary becomes a problem in diagnosis, urinary ACTH assay might be useful in addition to the already available assays of pituitary hormones.

TABLE 6. ACTH AND STEROIDS IN URINE OF PATIENTS WITH SURGICALLY PROVED CUSHING'S SYNDROME

	Patient No.	Name	Age & sex	ACTH (AAD, mg./100 Gm.)	17-OH-CS (mg./24 hrs.)	17-KS (mg./24 hrs.)	State of adrenals
Before operation	1	H.F.	11 M	101* 128*	7.5	14.2	Hyperplasia
	2	S.M.	12 M	45	5.8	9.1	Hyperplasia
	3	S.E.	25 F	−62	0.5†	4.5	Nodular hyperplasia
	4	K.Y.	26 F	37* 6*	25.0	3.9	Hyperplasia
	5	T.K.	28 F	−16 15	12.6 12.5	14.2 13.0	Hyperplasia
	6	Y.H.	28 F	80 −70 −67	3.5† 5.8	12.6 12.7	Adenoma left adrenal
	7	F.M.	33 F	125*	7.0	7.6	Hyperplasia
	8	S.K.	27 F	−28	9.5	31.0	Adenoma rt. adrenal
After operation	3	S.E.	25 F	90 66	3.9 —	6.1 —	10 days postop. 11 days postop.
	6	Y.H.	28 F	22 53 −22	0.3 0.8 4.2	3.9 4.5 4.1	10 days postop. 20 days postop. 90 days postop.

* 12-hour urine sample.
† Impaired renal function.

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RAPID METHOD FOR DETERMINATION OF ALDOSTERONE IN HUMAN URINE BY GLASS PAPER CHROMATOGRAPHY*

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ABSTRACT

A method for the determination of aldosterone in urine is described, which utilizes rapid chromatography on glass fiber paper. After acid hydrolysis, chloroform extracts of the urine are chromatographed on silica-gel columns, run on 2 glass paper chromatographic systems, acetylated, and again separated by 2 additional runs in separate glass paper chromatographic systems. Final determination of aldosterone is performed by measurement of alkaline fluorescence. The specificity and precision of the method, the recovery of steroid by this technic, and the range of values in normal man are presented. The advantages of glass paper chromatography are pointed out, not the least important of which is that each chromatographic step requires only fifteen minutes.

THERE are several quantitative procedures for the determination of urinary aldosterone which are of reasonable accuracy and specificity (1–5). The chemical methods now in use purify the urine extract and separate aldosterone from other steroids by means of paper chromatography in 3 to 4 runs, with the time required for one run varying from two to forty-eight hours. In addition, chromatograms of the Bush type have to be equilibrated for several hours prior to actual development.

The following procedure makes use of the rapid and simple technic of ascending chromatography of steroids on glass fiber paper (6, 7), thus eliminating the need for equilibration prior to chromatography and significantly reducing the time of development to fifteen minutes per run.

MATERIALS

1. *Silica gel*, activated, 100–200 mesh. Davidson Chemical Co., Baltimore, Md.
2. *Glass fiber filter paper* (No. 934-AH). H. Reeve Angel & Co. Inc., Clifton, N. J.

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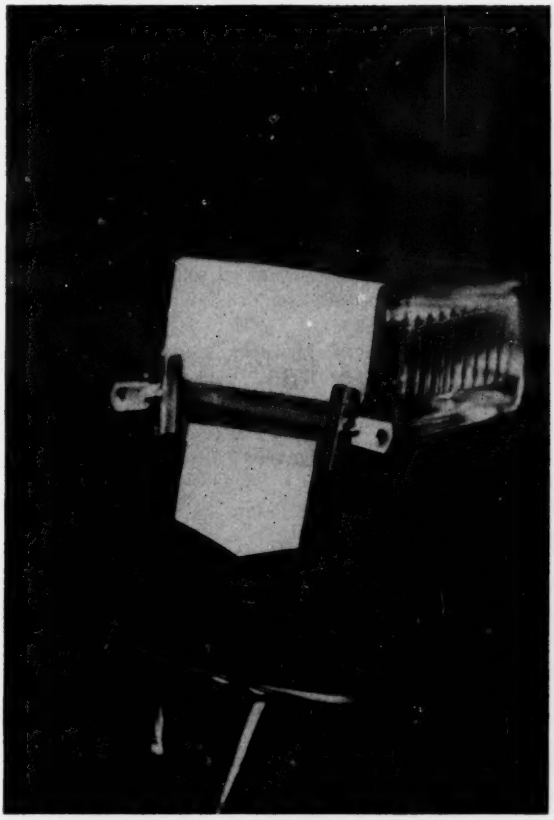

FIG. 1. Apparatus for descending elution of chromatograms.

Synthetic fiber paper sheet for chromatography. Each strip (8 cm.×20 cm.) is cleaned by heating for one hour at 1000°F., cooled, immersed in a solution of 0.1 *M* monobasic potassium phosphate, and dried in air.

3. *Acetone*, Baker, analyzed reagent. Four liters are refluxed with several small quantities of $KMnO_4$ until a violet color persists, dried over K_2CO_3 (anhydrous), filtered, and fractionated at 56–57°C.
4. *Benzene*. Four liters of benzene are shaken with approximately 50 ml. of concentrated H_2SO_4, and the acid layer removed. The benzene is washed twice with 100 ml. of distilled water, once with 50 ml. of 10 per cent Na_2CO_3 and finally with another 100 ml. of water. The benzene is dried over anhydrous $CaCl_2$, filtered and distilled. The fraction distilling at 80–81°C. is collected. The benzene is stored over metallic sodium.
5. *Tert.-butyl alcohol*. A 500-ml. volume of tert.-butyl alcohol, containing approximately 0.5 Gm. of dissolved sodium or potassium, is allowed to stand at room temperature for two weeks. It is then filtered through Florisil and distilled. This

reagent should remain colorless; if impurities are present, a yellow compound with green fluorescence will develop, requiring repetition of the purification procedure (8).

6. *Potassium tert.-butoxide.* Approximately 1 Gm. of potassium metal is scraped clean in a mortar containing petroleum ether, blotted between paper towels, and transferred to a flask containing tert.-butanol. After refluxing under nitrogen for two or three minutes, the molten potassium is washed by decantation with several portions of tert.-butanol and refluxed with 40 ml. of tert.-butanol until dissolved. An all-glass apparatus should be employed. The reagent is stored in a desiccator containing KOH. The butoxide concentration is estimated by titration with 0.1 *N* HCl. The fluorescence of the solution should not exceed that of a standard solution of quinine sulfate (0.1 μg. per ml.) in 0.1 *N* H_2SO_4. This reagent is stable for several weeks.
7. *Chloroform,* techn. Four liters of chloroform are shaken three times with approximately 200 ml. of concentrated H_2SO_4, washed twice with 200 ml. of distilled water, dried over phosphorus pentoxide and distilled in a nitrogen atmosphere. The fraction distilling at 61°C. is collected. Absolute ethyl alcohol 1:100 is added to the distillate as a stabilizer to prevent formation of phosgene.
8. *Acetone-chloroform,* 1+99.
9. *Acetone-chloroform,* 1+1.
10. *0.1 N NaOH.*
11. *Solvent I*—100 ml. of benzene mixed well with 1 ml. of N,N-dimethyl formamide (Matheson, Coleman and Bell Division, The Matheson Company Inc.).
12. *Solvent II*—100 ml. of cyclohexane mixed well with 27 ml. of acetone.
13. *Solvent III*—100 ml. of benzene mixed well with 0.025 ml. of N,N-dimethyl formamide.
14. *Solvent IV*—100 ml. of toluene mixed well with 1.0 ml. of ethyl acetate.
15. *Pyridine,* anhydrous, redistilled. This is refluxed over barium oxide for four to six hours and distilled in a fractionating column. The middle fraction boiling at 115°C. is collected.
16. *Acetic anhydride,* redistilled, is refluxed over calcium carbide for four to six hours, and distilled in a fractionating column. The middle fraction boiling at 139°C. is collected.
17. *Methylene chloride,* reagent grade, Baker, purified on a silica-gel column.

Reference standards:

a) Cortisol, or labeled[1] cortisol-4-C^{14}. If cortisol is used, a solution of approximately 5 μg. per 10 μl. is prepared. Cortisol serves as a qualitative reference standard, and exact concentration is therefore not necessary. Using cortisol-C^{14}, a solution of about 5000 c.p.m. per 100 μl. is prepared and quantitated for use in determining the efficiency of extraction from urine and elution of steroids from the silica-gel column and paper chromatograms.

b) Aldosterone.[2] Either the free alcohol or *d,l*-aldosterone-21-monoacetate may be used.

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18. *Blue tetrazolium* (BT) reagent, 0.1 per cent aqueous solution.
19. *Methanolic potassium hydroxide* solution. KOH, 14 per cent (w/v) in 60 per cent (v/v) purified methanol.
20. *Alkaline BT* reagent. One volume of reagent #18 is mixed with 9 volumes of reagent #19 immediately before use.
21. *Fluorometry equipment.* A Farrand fluorometer, model A is used. The upper portion of the slit in the cuvette holder is masked with tape, thereby decreasing the size of the aperture so that only 0.5 ml. of solution need be measured rather than 2.0 ml. as is usually required. This modification increases the sensitivity of measurement. Closely matched Pyrex test tubes, 10×75 mm., are used. The primary filter has a peak transmittance at 365 mμ and the secondary filter, a peak at 560 mμ. The same light aperture is used for all determinations (8).

PROCEDURE

Collection and storage of urine

Urine is collected in 24-hour specimens, and stored or refrigerated as recommended by others (4, 5).

Hydrolysis and extraction of steroid

Half the volume of a 24-hour urine is adjusted to pH 1.0 with concentrated HCl and kept at room temperature for forty-eight hours (4). To avoid formation of an emulsion (1), the urine is stirred gently for half an hour in a separatory funnel with 0.2 volume of chloroform, and this extraction is repeated three times. The 4 chloroform extracts are combined, washed three times with 0.1 volume of 0.1 *N* NaOH and twice with 0.1 volume of distilled water. The combined NaOH and water washings are back-extracted with an equal volume of chloroform. The chloroform extract is evaporated to dryness in a vacuum at 40°C. in an all-glass apparatus (4).

Purification on silica-gel (2)

Two columns (diameter 12 mm.) are prepared by suspending 6 Gm. of silica-gel in 10 ml. of chloroform, and adding 0.5 ml. of the suspension to each column. The dried urinary extract is dissolved in a small volume of chloroform, and equal portions are transferred to the columns. Not more than 20 mg. of residue should be applied to any one column. A 50-ml. volume of chloroform-acetone (99:1) is passed through each column and discarded. Each column is then eluted with 100 ml. of chloroform-acetone solution (1:1) and the combined eluates are evaporated to dryness.

First chromatogram

The dried urinary extract is dissolved in 1.0 ml. of chloroform and transferred quantitatively to prepared KH_2PO_4-impregnated glass fiber paper strips by streaking aliquots with a 50-μl. pipette along a transverse line perpendicular to the long axis of the paper and approximately 2 cm. above the lower edge. This line extends to within 1.0 cm. of either side. The paper is dried with a stream of warm air after application of each aliquot; usually 4 to 5 aliquots may be superimposed on one strip without overloading. Additional papers are similarly prepared. An average extract will require the use of about 4 sheets for this first phase of chromatography. If the extract is unusually concentrated, 2 or more additional sheets should be used. No more than 5 mg. of residue should be applied to one paper.

The chromatography chamber is simply a cylindrical glass jar, 30 cm. high and 15 cm.

in diameter, covered with a glass plate through which a hole has been bored for insertion of a stopper and a curved glass rod for suspension of the chromatograms in the solvent system (9).

Solvent I is used for the first chromatographic determination. The sheets are dipped into the solvent to a depth of about 5 mm. and the solvent rapidly ascends the paper, reaching the top in about fifteen minutes. The papers are then dried in a draftless oven at 40°C. The papers are cut across 3 cm. inside the front and 2 cm. inside the origin, and one end of the center section is tapered slightly in preparation for elution by the descending technic (Fig. 1). A clean paper strip is folded over the edge of a glass dish so that one half dips into the solvent and the other half hangs over the dish. This end is placed between two glass slides, the chromatogram to be eluted is inserted from below between the slides, and the slides are held together with paper clips. The glass dish is filled with sufficient acetone to provide a constant flow of solvent down the chromatogram at a rate of 1 drop per second. A 10-ml. volume of acetone is sufficient to elute one chromatogram completely. The eluates of the 4 papers are combined and evaporated to dryness.

Second chromatogram

The dried eluate is dissolved in 0.5 ml. of chloroform and applied to 2 sheets as previously described. A standard of 1.0 μg. of free aldosterone or cortisol is also run on one of the sheets, and the papers are developed in Solvent II. After drying, the standard section is cut from the chromatogram, sprayed with concentrated H_2SO_4 until thoroughly moistened, and then heated over a hot plate until the acid volatilizes. The steroid standard will appear as a gray-black spot on a white background, best visualized with transmitted light.

The chromatograms are cut 1 cm. above and below the corresponding aldosterone zone and eluted as described. If cortisol is used as a standard, a section 4 cm. long is cut out of the chromatogram from the lower border of the cortisol zone toward the origin, which will include the aldosterone zone.

Acetylation

The eluate is evaporated in a conical glass-stoppered tube, 100 μl. of pyridine and 100 μl. of acetic anhydride are added and the sample allowed to stand at room temperature in a desiccator for twelve hours. One ml. of 20 per cent ethyl alcohol is then added and the steroid extracted into 5 ml. of methylene chloride by vigorous shaking for twenty seconds and subsequent centrifugation. The aqueous layer is aspirated. The methylene chloride is washed twice with 1.0 ml. of distilled water and evaporated to dryness. An aldosterone standard (5 μg., calculated as free aldosterone) is acetylated at the same time.

Third chromatogram

The acetylated sample and standard are dissolved in 50 μl. of benzene, and each applied to separate papers. A reference standard of aldosterone diacetate is applied to a third paper and chromatographed with the others in Solvent III. After establishing the exact R_f of aldosterone diacetate by charring the reference standard with H_2SO_4, the corresponding zones on standard and extract papers are eluted and evaporated.

Fourth chromatogram

The standard, the extract, a reference standard, and a blank KH_2PO_4-impregnated paper strip are chromatographed in Solvent IV. The R_f of the reference standard is determined by charring this paper. The corresponding zones of equal areas from the stand-

ard, the extract and blank papers are eluted and evaporated. A 10-ml. volume of acetone is used for elution of each paper to minimize differences in background fluorescence.

Fluorometric determination (8)

Each sample is dissolved in 0.5 ml. of benzene, transferred quantitatively to the fluorometer cuvettes, and dried in a vacuum desiccator over concentrated H_2SO_4. Potassium butoxide reagent (0.5 ml.) is then added to each tube. The tubes are tightly stoppered with aluminum foil-covered corks, shaken, and allowed to stand for one hour at room temperature. The tubes are shaken again and read in the fluorometer.

Calculation

$$\frac{\text{fluorescence of sample} - \text{blank}}{\text{fluorescence of standard} - \text{blank}} \times \mu\text{g. free aldosterone in standard} \times \frac{\text{24-hr. urine volume}}{\text{volume of urine for extraction}} = \mu\text{g. aldosterone/24 hours.}$$

EXPERIMENTAL DATA

Deviation from linearity between fluorescence readings and amounts of aldosterone

Beginning deviation from linearity appeared between 7 and 8 μg. of aldosterone, in agreement with the findings of other authors (4).

Sensitivity of fluorometry

The lower sensitivity lies between 0.25 and 0.5 μg., dependent upon the blank fluorescence of the butoxide reagent (8). Multiple paper blanks demonstrated no significant variation in readings.

Completeness of separation

All urine extracts studied to date were divided into two equal parts after the third chromatogram, and one portion was quantitatively applied to another chromatogram within an area 1 cm. in diameter. The chromatogram was then developed in System IV and drawn through freshly prepared alkaline BT reagent.

In each instance only one blue spot was observed in the aldosterone diacetate zone; in 3 samples, unidentified reactive material was also present at the origin.

Efficiency of steroid extraction

Equal quantities of cortisol-4-C^{14} were added to a urine sample and also to an equivalent volume of chloroform. Extraction was carried out for thirty minutes as previously described and the final volume of the chloroform extract was adjusted to the original volume by addition of more chloroform. Equal quantities of the chloroform standard and the extract were counted in a gas-flow counter, and the efficiency of extraction of

isotopic cortisol from urine was calculated. In 7 experiments, the results varied between 79.5 per cent and 83.0 per cent, with a mean of 81.8 per cent. Prolongation of the period of extraction to two hours did not yield significantly higher recoveries. Since cortisol and aldosterone resemble each other in their polarity and chromatographic properties, the conclusion seems justified that the percentage of free aldosterone extracted from urine by this technic would be similar to that for cortisol.

Efficiency of elution from silica-gel columns and from glass fiber paper chromatograms

Almost 100 per cent of the cortisol-C^{14} added to urine extracts was recovered from the silica-gel columns, and similar quantitative recovery of this compound from chromatograms was demonstrated for the technic of descending elution using 10 ml. of acetone. In the technic of elution of the chromatograms, the flow of acetone down the paper strip must be kept uniform in order to achieve quantitative recovery of the steroid. This is best accomplished by keeping the level of the liquid between the slides horizontal, by proper adjustment.

Influence of impurities on R_f values

The R_f values of pure steroid standards have been shown to be reasonably constant and not to be dependent upon equilibration, temperature and humidity to an extent that would interfere with the method. Impurities in the first chromatogram, however, will increase the R_f of steroids significantly. Pure cortisol-C^{14}, identified with the use of a chromatogram scanner, has an R_f of 0.48 in benzene: dimethyl formamide (100:2), but the same compound added to urinary extracts will run close to the front on the first chromatogram in this solvent. Chromatography in 1.0 ml. of dimethyl formamide to 100 ml. of benzene (Solvent I) will slow the R_f of the steroid standard to 0.26, and the steroid added to urinary extracts will have an R_f of approximately 0.50, dependent upon the total quantity of impurities present.

Since cortisol C^{14} has always been found to run close to the center of the first chromatogram in Solvent I and significant streaking of the compound has never been observed even when added to heavily laden extracts, the described technic for removal of those areas of the paper close to the origin and the front may be utilized to eliminate most of the impurities without loss of aldosterone. In subsequent chromatographic steps, the remaining impurities should not influence R_f if the sample is run on the suggested number of paper sheets.

Recovery of aldosterone added to urine

In 5 experiments, 10-μg. portions of free aldosterone were added to urine after acid hydrolysis. Recovery varied between 78.0 per cent and 82.0 per cent, with a mean recovery of 80.5 per cent. Duplicate determinations of aldosterone in 4 normal urines showed an average error between duplicates of 6.2 per cent, with a range of from 0 to 14 per cent.

Normal values

Urinary aldosterone levels were determined in 16 adult males and 14 adult females in apparent good health. Aldosterone excretion ranged from 3.0 to 16.0 μg., with no significant difference apparent between the sexes.

DISCUSSION

This method for separation of aldosterone from urinary extracts using glass fiber paper chromatography has several advantages over conventional filter paper methods, without sacrifice of the specificity and accuracy of paper chromatographic technics:

a) Development is rapid and one chromatogram requires no more than fifteen to twenty minutes for completion.
b) Localization of steroid standards by the technic of charring with sulfuric acid is rapid and simple to perform.
c) Time-consuming equilibration of chromatograms is not required. Temperature and humidity are of little influence. The chromatography does not require expensive equipment.
d) Approximately 30 aldosterone determinations can be performed in one week by one technician.

The advantages of quantitation by alkaline fluorescence have been discussed previously (10).

In none of the urines studied to date have we been able to detect a steroidal compound running close enough to aldosterone to interfere with its quantitative determination. The normal urinary aldosterone values obtained by this method are in good agreement with those obtained by other methods previously reported.

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FAILURE OF THIOUREA TO PREVENT HORMONE SYNTHESIS BY THE THYROID GLAND OF MAN AND ANIMALS TREATED WITH THYROTROPIN*†

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ABSTRACT

The effect of thyrotropin (TSH) upon the thiourea-inhibited thyroid gland was investigated in man and in animals. A single 500-mg. oral dose of thiourea virtually suppressed the thyroidal 24-hour uptake of radioiodine (I^{131}) in 23 patients. Treatment of some of the same subjects with 30 units of TSH before the administration of 500–750 mg. of thiourea resulted in threefold to sixfold increments in I^{131} uptake. The TSH-induced increment of thyroidal radioactivity in association with large doses of thiourea was not great and represented only "trapped" iodide, whereas the elevated uptake values noted in association with smaller doses of thiourea usually represented organic iodine. A 70-mg. dose of thiourea injected into guinea pigs before the administration of radioiodine prevented the formation of endogenous thyroxine-I^{131}, as shown by chromatography of the thyroid hydrolysates. However, in animals receiving 6 to 9 units of TSH before the administration of identical doses of thiourea and radioiodine there was no impairment of thyroxine synthesis.

PREVIOUS studies in euthyroid subjects have shown that thyrotropin may restore the ability of the thiocyanate-inhibited thyroid gland to incorporate iodine, presumably through an effect on the iodide-concentrating mechanism (1, 2). The present studies were carried out to ascertain the effect of thyrotropin (TSH) on the organic binding of iodine by the thyroid gland in the presence of thiourea.

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MATERIAL AND METHODS

Patients

Twenty-three convalescent patients, hospitalized for conditions unrelated to thyroid disease, participated in the study; more detailed studies were carried out in 11 of the 23. The individual data on these 11 subjects are recorded in Table 1, and the combined values are shown in Figure 1.

The technique used for repeated tracer radioiodine uptake determinations in the same patient has been outlined in a previous publication (1). Following the baseline de-

TABLE 1. ABSOLUTE CHANGES IN THYROIDAL RADIOACTIVITY IN TSH-TREATED PATIENTS AFTER ADMINISTRATION OF PERCHLORATE OR THIOCYANATE
(RaI uptake, % of dose)

Patient	Thiourea, 500 mg.	TSH plus 500 mg. thiourea	TSH plus 750–1000 mg. thiourea	Radioactivity remaining after	
				$KClO_4$	KSCN
E.A.*	5.6	25.1			5.3
R.B.	1.9		3.7		0
H.B.	3.1		6.6	3.6	
D.C.	4.1		13.9	13.8	
E.C.	2.1		6.9	6.1	
G.J.	6.0		7.8		2.5
G.J.			8.9	3.6	
M.M.	3.6		10.4		1.3
E.Z.	2.6	20.3		21.2	
C.D.	3.1	13.0		10.5	
G.D	0		6.9		2.1
K.G.	5.0	20.6		20.3	

* In the group pre-treated with TSH and receiving 500 mg. of thiourea, Patient E.A. was the only one whose thyroidal radioactivity was almost completely discharged by 2.5 Gm. of thiocyanate. Note the lesser response to TSH in the group receiving the higher dose of thiourea, and the frequency of thyroidal discharge of radioactivity by perchlorate or thiocyanate. The response to 300 mg. of perchlorate appeared to be similar in magnitude to the response to 2.5 Gm. of thiocyanate with respect to the amount of thyroidal radioactivity released, as shown in Patient G.J.

termination of the 24-hour thyroidal radioiodine uptake (RaI), the subject received a single 500-mg. dose of thiourea (TU). Three to four hours later an oral tracer dose of 50 to 100 μc. of carrier-free radioiodine (I^{131})[1] was administered, and the thyroidal uptake of I^{131} was measured twenty-four hours from the time of thiourea ingestion. Then a dose of 10 U.S.P. units of thyrotropin[2] was administered intramuscularly each day for three consecutive days. On the third day the subject was given a single oral dose of thiourea (500–750 mg.), followed after four to five hours by a tracer dose of radioiodine. Twenty-

[1] Abbott Laboratories, Oak Ridge, Tenn.

[2] Thytropar, Armour Pharmaceutical Co., Kankakee, Ill.

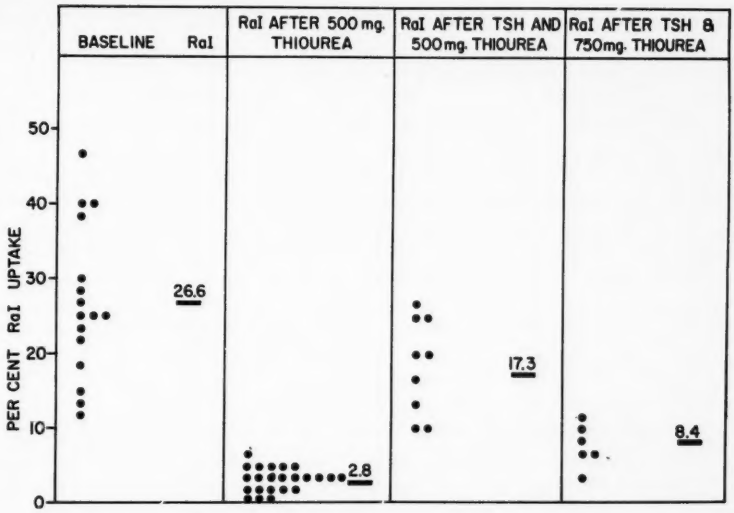

FIG. 1. Effect of thiourea upon the 24-hour thyroidal uptake of radioiodine before and after administration of TSH. The effect of TSH upon the uptake values in the patients receiving 500 mg. of thiourea was significantly greater than that in the patients receiving 750 mg. of thiourea ($P < 0.01$).

four hours from the time of thiourea ingestion the thyroidal uptake of I^{131} was determined, and at the same time either 2.5 Gm. of potassium thiocyanate or 300 mg. of potassium perchlorate was administered orally. Four hours later thyroidal radioactivity was measured again. In 2 patients, the thiourea and I^{131} were administered a third time several days after the TSH effect was no longer apparent.

Animals

Separate experiments were carried out on different days using groups of 10 to 12 male guinea pigs[3] (250–300 Gm.) in each experiment. The animals were caged under identical conditions, receiving Rockland guinea-pig diet, vegetables, and water without restriction. Half of the animals in each group, drawn at random, served as controls.

The TSH was dissolved in physiologic saline (10 U.S.P. units of TSH per ml.). All injections were made intraperitoneally, each animal receiving 2.0 to 3.0 units of TSH daily for three consecutive days. An equal volume of saline which did not contain TSH was administered to the control animals at the same time. On the final day of the TSH injections, each animal received a single 70-mg. dose of thiourea dissolved in physiologic saline, followed in three hours by 75 to 150 μc. of carrier-free I^{131}. The next day the animals were exsanguinated under ether anesthesia by cardiac puncture, using a heparinized syringe. The thyroid glands were excised, cleaned, and dropped into a glass tissue-grinder containing 0.5 ml. of distilled water and small amounts of thiourea and pancreatin. After the thyroid glands had been homogenized by hand, the grinders were placed in a water bath at 37° C. for forty-eight hours.

Following digestion in the grinder, an aliquot of approximately 10–20 lambda of

[3] Samuel E. Rice, Saugus, Mass.

thyroid hydrolysate was applied along a 2-cm. line to Whatman No. 1 chromatography paper. Chromatograms were run in an ascending solvent system of butanol, acetic acid and water (75:10:15) for eighteen to twenty-four hours at room temperature. The zones of radioactivity along the paper strips were located by means of autoradiography on No-Screen x-ray film. Identification of the thyroid fractions was made by comparing them with known radioactive and stable carrier materials.

A 5-ml. portion of cold TCA (trichloroacetic acid) was blown into each 1-ml. aliquot of the guinea-pig plasma contained in centrifuge tubes. After the finely dispersed precipitate had settled, the tubes were centrifuged and the supernatant solution was decanted. The precipitate was washed twice more with 5-ml. volumes of 3 per cent cold TCA. After the final washing, the radioactivity in the precipitate was measured in a well-type scintillation detector. The plasma PBI-I^{131} concentration (per cent of the administered dose of radioiodine per liter of plasma) was calculated using appropriate I^{131} standards.

RESULTS

The thyroidal radioiodine uptake values in the patients receiving thiourea, before and after TSH, are summarized in Figure 1. The uptake values after TSH and thiourea do not necessarily reflect the hormonal incorporation of radioiodine. As may be noted in Table 1, it was possible to determine the extent of organic binding of the radioiodine in only 4 of the 9 patients receiving the 500-mg. dose of thiourea. One 500-mg. oral dose of thiourea was almost completely effective in suppressing the 24-hour radioiodine uptake of the thyroid gland. The same patients, when pre-treated with TSH, exhibited an appreciable degree of thyroid function in the presence of thiourea. Furthermore, the effect of TSH was more marked in the patients receiving the 500-mg. dose of thiourea than in those receiving the 750-mg. dose.

Whether or not the radioactivity within the thyroid gland represented hormonal iodine under these circumstances was determined by the procedures illustrated in Figures 2 and 3. As shown in Figure 2, the administration of 300 mg. of potassium perchlorate failed to release any of the thyroidal radioactivity following the TSH-augmented I^{131} uptake, thus demonstrating organic binding of the radioiodine. A different response to both TSH and perchlorate was elicited in the patient in Figure 3. There was virtually no enhancement of the radioiodine uptake in response to TSH, and the perchlorate produced an immediate discharge of the thyroidal radioactivity. Hence, in some instances hormonal iodine was formed in response to TSH, whereas in others only inorganic iodide was found.

The extent to which the trapping of iodine by the thyroid gland occurred without subsequent organic binding is indicated in Figure 4. There was a reduction in the mean radioiodine uptake in the TSH-treated patients following either thiocyanate or perchlorate. The absolute changes in uptake for each patient following administration of thiocyanate or perchlorate are shown in Table 1. Examination of these values reveals several points of

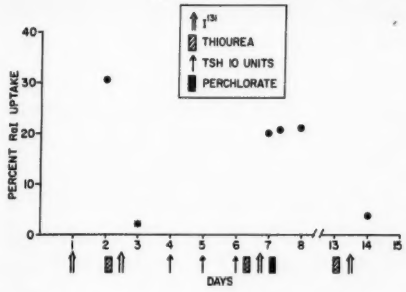

FIG. 2. Repeated tracer radioiodine studies in the same patient receiving, at various times, thiourea, TSH and potassium perchlorate. The augmented RaI uptake after administration of TSH remained unchanged after the administration of perchlorate.

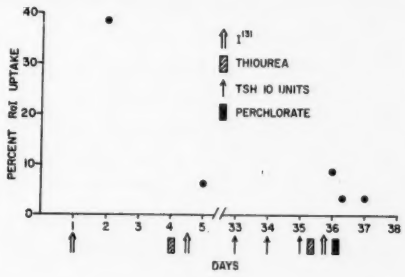

FIG. 3. Repeated tracer radioiodine studies in a patient showing lack of response to TSH after a 750-mg. dose of thiourea. Note the reduction in thyroidal radioactivity following 300 mg. of potassium perchlorate.

interest: 1) In patients receiving 750–1000 mg. of thiourea, the administration of TSH caused little or no augmentation of radioiodine uptake by the thyroid. 2) In 5 of the 7 patients in the same group, the thyroid concentrated I^{131} in response to the TSH, but there was only limited organic binding of the I^{131}, as indicated by reduction in the uptake after administration of perchlorate or thiocyanate. 3) With the single exception of Patient E.A.,

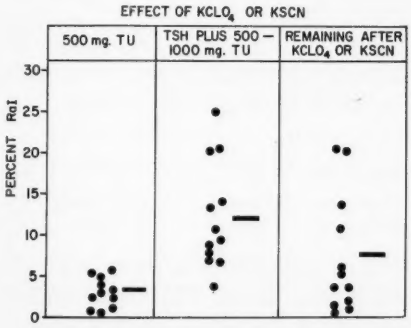

FIG. 4. Effect of potassium thiocyanate or perchlorate upon the RaI uptake in the TSH-treated thyroid gland. The mean radioiodine value for all dose levels of thiourea was reduced following the administration of 2.5 Gm. of potassium thiocyanate or 300 mg. of potassium perchlorate.

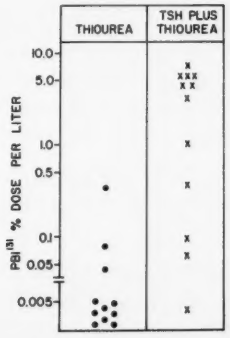

FIG. 5. Effect of TSH upon the plasma PBI-I^{131} concentration in guinea pigs receiving thiourea.

the subjects receiving the smaller dose of thiourea responded to the TSH with increased thyroidal incorporation of I^{131} which was not discharged by thiocyanate or perchlorate.[4]

The results of the studies in guinea pigs presented additional evidence that the thiourea-inhibited thyroid gland can be induced to synthesize hormone following administration of TSH. Figure 5 shows the marked increase in the plasma concentration of PBI-I^{131} in animals treated with TSH before receiving thiourea, as compared with the controls receiving only thiourea. It is interesting that in the former there was considerable

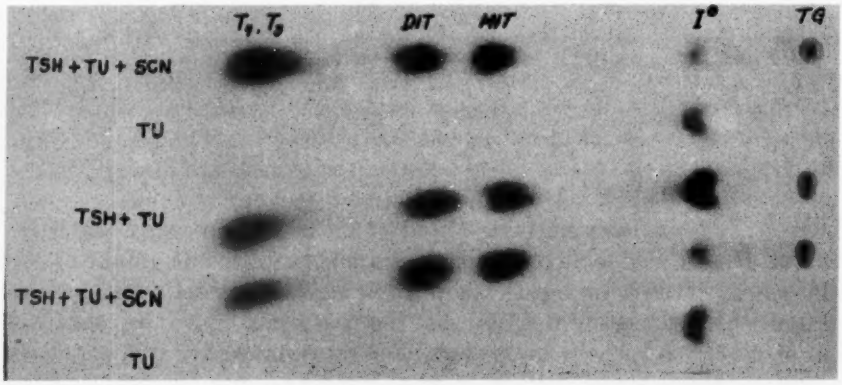

FIG. 6. Autoradiograph of chromatogram of guinea-pig thyroid hydrolysates. Note the absence of organic iodine in animals receiving only thiourea (TU), whereas in animals receiving TSH and thiourea there was no impairment of thyroxine synthesis. Potassium thiocyanate in the TSH-treated animals reduced the iodide peaks but did not affect production of hormonal iodine. TG = thyroglobulin (origin); I^- = iodide; MIT = monoiodotyrosine; DIT = diiodotyrosine; T_3 = triiodothyronine; T_4 = thyroxine.

variability in the magnitude of the response to the TSH, some of the PBI-I^{131} values in TSH-treated guinea pigs being no different from those in the control animals.

The chromatograms made from the thyroid hydrolysates clearly indicate the presence of hormonal iodine in the animals receiving TSH. As shown in Figure 6, the thiourea completely prevented the formation of thyroxine in most of the control guinea pigs, but the thyroids of animals treated with TSH and thiourea exhibited appreciable concentrations of organic iodine. Occasionally, chromatograms of thyroid hydrolysates from the control animals also revealed the presence of hormonal iodine.

[4] In Patient C.D., perchlorate had only a slight effect.

DISCUSSION

The ability of a single 500-mg. oral dose of thiourea to prevent the uptake of radioiodine by the normal thyroid for twenty-four hours was described earlier by Stanley and Astwood (3). The studies reported here confirm their observation and show that the thiourea effect may be partially abolished by thyrotropin. The dose level of thiourea appeared to influence the magnitude of the response to TSH. The TSH-induced increment of thyroidal radioactivity in association with high doses of thiourea was not great and represented only "trapped" iodide. With smaller doses of thiourea the elevated uptake values in response to TSH usually represented organic iodine (Table 1).

The data shown in Table 1 may also be interpreted in a different manner. In every instance, thiocyanate produced an immediate release of almost all of the radioactivity within the thyroid gland. Perchlorate, on the other hand, either failed completely to reduce the level of thyroidal radioiodine or elicited less response. The conclusion may be drawn that the thiocyanate was much more potent in its ability to discharge iodide than was the perchlorate. However, it should be emphasized that, with the single exception of patient E.A. (Table 1), the thiocyanate was administered to patients receiving the larger doses of thiourea. Hence, one might be equally justified in concluding that the conditions were not precisely the same, and that the patient receiving thiocyanate was more likely to have a larger fraction of inorganic iodide within his thyroid gland than was the patient receiving perchlorate. That the actions of perchlorate and thiocyanate may have been similar in magnitude is supported by the results in Patient G.J. (Table 1). This patient had been treated with TSH and a single 500-mg. dose of thiourea before he received thiocyanate or perchlorate.

The means by which TSH was able to restore partial function to the thiourea-inhibited thyroid gland is not easily explained. Maloof and Soodak (4) demonstrated that S^{35}-labeled thiourea is accumulated rapidly in the thyroid glands of normal rats and quickly metabolized, the labeled sulfur atom being converted chiefly to S^{35}-sulfate. When thiourea-S^{35} was administered to hypophysectomized rats, the rate of metabolism of the labeled material was greatly reduced. On the basis of these findings one might deduce that TSH accelerates the metabolic degradation of thiourea.[5] Further evidence for this, of a more indirect nature, may be gathered from the report showing that TSH hastens the intrathyroidal metabolism of thiocyanate-S^{35} in normal guinea pigs and that S^{35}-labeled sulfate is the chief

[5] Recent studies in this laboratory have shown that TSH effects a rapid intrathyroidal degradation of thiourea-S^{35} in guinea pigs.

product of degradation (5). Since the sulfur atom of thiourea is also oxidized to sulfate, one might postulate a common metabolic pathway in both instances.

The finding that TSH may induce organic incorporation of iodine in the thiourea-inhibited thyroid gland may have some bearing upon the interpretation of experimental data pertaining to thyroid-serum ratios. Since it appears that an antithyroid agent may be ineffective in completely preventing the organic binding of radioiodine when TSH is used, calculations of the size of the thyroidal iodide trap based on thyroid-serum ratios should take into consideration the possibility of organically bound radioiodine.

Whether or not these studies have any clinical significance has yet to be determined. A relationship might be suggested between the foregoing findings and the persistent thyroidal uptake of radioiodine in some hyperthyroid subjects receiving antithyroid compounds.

Acknowledgment

The authors wish to express their appreciation to Dr. E. B. Astwood for supplying his time and his ideas so generously throughout the course of these studies.

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FORMATION OF ANDROGENS BY HUMAN ARRHENOBLASTOMA TISSUE *IN VITRO**†

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ABSTRACT

Arrhenoblastoma tissue slices were incubated *in vitro* with progesterone-4-C^{14}. The radioactive products isolated were 20α-hydroxy-4-pregnen-3-one, 20β-hydroxy-4-pregnen-3-one, 17-hydroxyprogesterone, 4-androstene-3,17-dione and testosterone. No estrogen was formed, nor was there evidence of the presence of the enzyme 11β-hydroxylase. Considerable significance is attached to the demonstration of the biosynthesis, in this tumor tissue, of the very potent androgen testosterone, as affording a ready explanation for the virilism associated with the syndrome and the frequent finding of normal (or slightly elevated) urinary 17-ketosteroid levels.

THE presence of androgens and their formation in ovarian tissue (1, 2) has long held the attention and curiosity of investigators in endocrinology (3). The recent chemical studies on the steroids of the human ovary by Zander and co-workers have resulted in the isolation of the androgenic steroid androstenedione (4-androstene-3,17-dione) from human follicles and corpora lutea (4). The pronounced virilizing effects of ovarian arrhenoblastoma have been little understood (5–9), and in the light of the aforementioned recent findings it would seem likely that androgen formation by this ovarian tumor might resemble the biosynthesis of testosterone and androstenedione from progesterone by normal male gonads (10). The present case afforded an opportunity to assess this point and this report deals with the results of incubation *in vitro* of arrhenoblastoma slices with progesterone-4-C^{14}.

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‡ Investigator of the Howard Hughes Medical Institute.

CLINICAL ASPECTS

A 23-year-old white unmarried woman was first seen in March 1955 at the Mount Sinai Hospital Out-Patient Diagnostic Center with complaints of hoarseness and amenorrhea of six months' duration. She had noted enlargement of the neck, nervousness, irritability and dryness of the mouth, and she had always been prone to excessive perspiration. The basal metabolic rate, determined elsewhere, was said to be +23 per cent. The findings on physical examination at the time of her first visit to the clinic were essentially normal, apart from a few acneform pustules over the posterior thorax and a slight increase in the amount of the hair in the region of the temples and chin. The thyroid was not enlarged and there was no tremor. The eyes were slightly prominent. Laryngeal examination showed nothing abnormal. On gynecologic examination, the clitoris was found to be approximately six times the normal size. The uterus was normal in size and position and no adnexal masses were felt.

The laboratory examinations, including routine urinalysis, complete blood count, erythrocyte sedimentation rate, glucose tolerance test, serum cholesterol level, and blood Wassermann and Kahn tests yielded values within the normal range. The basal metabolic rate was 0 per cent. The findings on roentgen examination of the chest, skull and abdomen were not remarkable. The urinary neutral 17-ketosteroids (11) were 9.5 mg. per twenty-four hours and the formaldehydogenic steroids (12) were 0.79 mg. per twenty-four hours. In vaginal smears, more than half the cells were cornified. Gynecography was performed. The body of the uterus and the left ovary were normal in size. On the right side a homogeneous globular dense mass was thought to represent an enlarged right ovary.

In the interval between a visit to the diagnostic clinic in September 1955 and the time of admission to the hospital on March 6, 1956, the patient noted weakness and progressive hirsutism of the face and chest, and amenorrhea persisted. On physical examination the findings were essentially similar to those noted previously, except for more marked hirsutism of the chin and cheeks. On pelvic examination, the right ovary appeared to be slightly enlarged.

In March 1956, the urine was normal. The blood hemoglobin level was 13.4 Gm. per 100 ml. The red blood cell count was 4.5 million per cu. mm., and the white blood cell count was 10,000 per cu. mm. with a normal differential count. The erythrocyte sedimentation rate was 6 mm. per hour. The concentration of blood urea nitrogen was 17 mg. per 100 ml., fasting blood sugar 97 mg. per 100 ml., serum chloride 95 mEq. per liter, carbon dioxide 27.6 mEq. per liter, calcium 11.5 mg. per 100 ml., phosphorus 2.7 mg. per 100 ml., sodium 141 mEq. per liter, potassium 4.7 mEq. per liter, cholesterol 249 mg. per 100 ml., bilirubin 0.87 mg. per 100 ml., uric acid 5.8 mg. per 100 ml., total protein 7.7 Gm. per 100 ml., albumin 4.5 Gm., and globulin 3.2 Gm. per 100 ml. The electrocardiogram was normal. The urinary excretion of neutral 17-ketosteroids was 11.4 mg. per twenty-four hours. The thyroidal uptake of a tracer dose of I^{131} was 48 per cent at the end of twenty-four hours and the total plasma I^{131} level was 0.07 per 100 ml. at the end of seventy-two hours. The basal metabolic rate was +17 per cent. The blood group was Rh positive. Vaginal smears, made over a period of several days, revealed an estrogen deficiency. Retrorectal gas insufflation showed that both adrenals were normal in size and configuration. Gynecography again revealed a large right ovary.

Operation. Laparotomy was performed. Several hundred milliliters of clear straw-colored fluid were found in the peritoneal cavity. The uterus was somewhat hypoplastic. The right ovary was markedly enlarged and contained a firm mass 4 cm. in diameter. Right oophorectomy was performed. The left ovary was approximately 4 cm. × 2 cm. in size and a triangular wedge was excised for histologic study.

Pathology and histology. Pathologic examination of the *right* ovarian mass revealed it to be irregularly lobular and yellow in color, the lobules being separated by strands of fibrous tissue. The surrounding ovarian tissue was slightly edematous and contained multiple small cysts filled with clear fluid.

Histologically, the tumor was highly differentiated, the cells being arranged as groups of pseudotubules without lumens. These were separated by an interlacing connective tissue stroma which was quite cellular in areas but did not contain cells resembling hilus or Leydig cells (Fig. 1). The cytoplasm of the tumor cells was eosinophilic and granular. Their nuclei were elongated and tended to be arranged in a basal position perpendicular to the basement membrane. Several groups of tumor cells showed varying degrees of cytoplasmic vacuolization. In comparison with the appearance of the dominant type of tumor cells, the nuclei of these latter cells were more vesicular, somewhat larger and more ovoid. They did not appear to lie free in the stroma but seemed to be confined to portions of the pseudotubules where they were enclosed by basement membrane. The diagnosis was arrhenoblastoma.

The biopsy specimen of the *left* ovary showed numerous primordial follicles, a rare maturing follicle containing an oocyte within its cumulus, a few atretic cystic follicles with perifollicular theca-cell proliferation and luteinization, and an old corpus albicans.

The postoperative course was uncomplicated. When seen eighteen days postoperatively, the facial hair was slightly decreased in amount and the clitoris appeared to be slightly smaller. Twenty-four days postoperatively, a 4-day painful menstrual period began, with less than the usual flow. The vaginal smear indicated a distinct estrogenic effect, and endometrial biopsy showed a secretory endometrium. The basal body temperature record confirmed ovulation on the fourteenth day of the cycle. On April 21, 1956, one month after surgery, the urinary neutral 17-ketosteroids were 9.2 mg. per twenty-four hours. Subsequent menstrual periods were regular and ovulatory. However, prolonged follow-up revealed persistence without regression of the hirsutism and the enlarged clitoris.

BIOCHEMICAL STUDY

Incubation

Portions of the tumor obtained at operation were immediately chilled, and within forty-five minutes were sliced on a Stadie-Riggs hand microtome and distributed in four incubation flasks. These flasks contained as their final contents 1.8 Gm. of tissue slices, 25 ml. of fresh human serum, 0.24 ml. of 0.1 *M* fumarate solution, 10 mg. of glucose and 500 units of sheep pituitary gonadotropin (mixed FSH and LH, Armour). To each of two flasks were added sodium acetate-1-C^{14} (1 mc.) and cholesterol-4-C^{14} (2 mg. containing 4×10^6 c.p.m.); subsequent chemical analysis of the contents of these two flasks failed to reveal any significant formation of radioactive steroids from either substrate. To the third flask was added 1.2 mg. of chromatographically homogeneous progesterone-4-C^{14} (5.5 μc.) in 0.25 ml. of ethyl alcohol. Incubation was carried out with shaking for three hours at 37° C. in an atmosphere of 95 per cent O_2 and 5 per cent CO_2. The contents of the flasks were frozen until analysis.

Analysis

In order to facilitate the isolation of the small amounts of anticipated radioactive products formed from progesterone-4-C^{14}, carrier steroids were added to the incubation flask at the time of analysis. The compounds and their amounts are listed in Table 1.

The contents of the flask (tissue, medium and carrier steroids) were subjected to extraction, purified, and separated into phenolic and neutral polar ketonic lipid fractions by a procedure designed to remove as much extraneous nonsteroidal material as

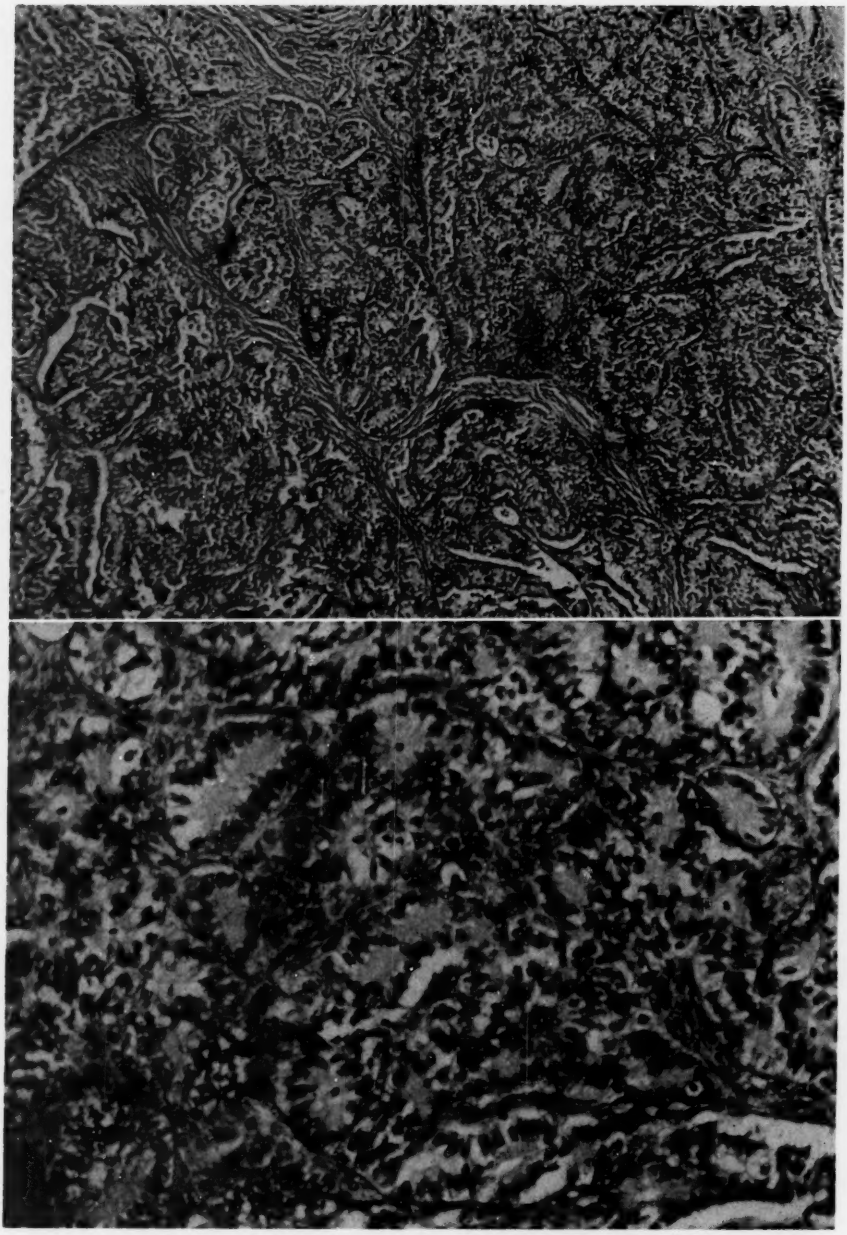

FIG. 1. Arrhenoblastoma. The microscopic picture is that of a highly differentiated tumor. Note the strong resemblance to normal testicular tubular structures and the absence of interstitial (Leydig) cells. (Magnification: top section, reduced from ×116; lower section, reduced from ×392.)

possible. The details of the methods are described elsewhere (13). The final phenolic fraction weighed 23.6 mg. and contained 38,800 c.p.m.; the neutral ketonic fraction weighed 83 mg. and contained 1.5×10^6 c.p.m. The neutral nonketonic fraction contained 138,000 c.p.m. and was not studied.

The phenolic fraction was separated on paper chromatograms in toluene/propylene glycol and the resulting 3 estrogens were individually purified by countercurrent distribution in the system toluene/1 *N* sodium hydroxide (14) and by partition chromatography on 0.4 *N* sodium-hydroxide impregnated Celite-benzene columns adapted from Bauld (15). All 3 estrogen carriers were found to be devoid of radioactivity.

The neutral ketonic fraction was chromatographed on filter paper in ligroin/propylene glycol for forty-eight hours (13). An unusual but fortuitous result was obtained

TABLE 1. PRODUCTS FOUND FOLLOWING INCUBATION OF PROGESTERONE-4-C^{14} WITH ARRHENOBLASTOMA TISSUE SLICES

Compound	Amt. added (mg.)	Radioactivity		Per cent of substrate*
		Spec. activity	Total c.p.m.	
Estrone	10.6	0	nil	0
Estradiol-17β	10.0	0	nil	0
Estriol	12.7	0	nil	0
17-Hydroxyprogesterone	20	12,000	240,000	4.8
Androstenedione	20	2,000	44,000	0.9
Testosterone	20	319	6,380	0.1
20β-Hydroxy-4-pregnen-3-one	10†	271	> 2,710	>0.05
20α-Hydroxy-4-pregnen-3-one	10†	2,600	>26,000	>0.5
11β-Hydroxy-4-androstene-3,17-dione	20	0	nil	0

* Calculated from the total radioactivity of the progesterone-4-C^{14} added as 5×10^6 c.p.m. (45 per cent counting efficiency).

† These were added as carriers late in the separation procedure, and the total accumulation of c.p.m. is not comparable to others

wherein all carrier steroids were found on the chromatograms (16). A portion of one of these was scanned for radioactivity; the results are shown in Figure 2, indicating at least 7 radioactive areas in addition to that of the recovered radioactive substrate (Zone I). Each of the zones of carrier steroids with its associated radioactivity was eluted and purified according to methods already described (13). The characterization of Zones III and IV, which had not been anticipated (and therefore for which no carrier steroids were added initially), is described subsequently. The radioactivity associated with each steroid carrier after demonstration of radiochemical purity is listed in Table 1.

Androstenedione (Zone II). This material, after crystallization, was radiochemically pure (specific activity,[1] 2200) as shown by the fact that 3.8 mg. diluted with 18 mg. of additional carrier and crystallized from ethyl acetate yielded crystals of weight 11.2 mg. (m.p. 170–170.5° uncorrected) and specific activity 365, with a mother liquor residue of weight 10.7 mg., specific activity 390. Formation of the oxime did not change the specific activity of the crystalline product.

17-Hydroxyprogesterone (Zone V). The material was crystallized several times from

[1] Radioactivity was determined in a flow-gas counter operating at 45–50 per cent efficiency; samples were plated in aluminum dishes at infinite thinness. Specific activity is expressed as counts per mg. per minute. In cases of specific activity of derivatives, values are not corrected for changes in molecular weight unless otherwise specified.

methanol, to yield 17-hydroxyprogesterone of specific activity 12,000. A 4-mg. portion was diluted with 10.0 mg. of additional carrier and crystallized from methanol to yield a mother liquor residue weighing 3.3 mg. and crystals 11.9 mg., with specific activities of 2980 and 3350, respectively. Formation of the oxime from the crystals did not change the specific activity from the calculated value.

Testosterone (from Zone V). Obtained as the acetate (13), this compound had a specific activity of 294 (corrected to the free compound, 319). Dilution with crystalline testosterone acetate and crystallization from hexane yielded a final mother liquor and crystals with specific activities of 50 and 50.5, respectively. Deacetylation and crystallization did not change the specific activity.

11β-Hydroxy-4-androstene-3,17-dione (Zone VI). This steroid was chromatographed in toluene/propylene glycol (five hours) and yielded 8.6 mg., coincident with a zone of radioactivity containing 37,700 c.p.m. On chromatography in ligroin/propylene glycol (ninety-six hours) the steroid carrier and the radioactivity were distinctly separated, the radioactivity being located as a discrete zone of lower mobility than that of the carrier steroid. Further attempts to characterize this radioactive product failed; it was found, however, to have the same chromatographic mobility as 6β-hydroxy-4-androstene-3,17-dione in ligroin/propylene glycol (16). The carrier steroid 11β-hydroxy-4-androstene-3,17-dione was not entirely devoid of radioactivity, but several crystallizations from hexane/acetone yielded consecutive products with steadily diminishing specific activity, indicating radiochemical contamination. In the absence of evidence of radiochemical purity, it was concluded that, in this experiment, radioactive 11β-hydroxy-4-androstene-3,17-dione was not formed from progesterone-4-C^{14}.

20β-Hydroxy-4-pregnen-3-one (Zone III). The zone of radioactivity immediately slower than androstenedione (Fig. 2) was eluted and 10 mg. of crystalline 20β-hydroxy-4-pregnen-3-one added. The mixture was rechromatographed and crystallized (acetone) to yield crystals (m.p. 170–171.5° corrected) and a mother liquor residue, each with a specific activity of 271. Both were combined, acetylated and recrystallized from hexane, yielding crystals of 20β-acetoxy-4-pregnen-3-one (m.p. 160–160.5° uncorrected) with a specific activity of 255.

20α-Hydroxy-4-pregnen-3-one (Zone IV). The material eluted from this area (Fig. 2) was diluted with 10 mg. of authentic 20α-hydroxy-4-pregnen-3-one and purified in the same fashion as described for the 20β-hydroxy epimer. Radioactivity in the crystals of the free compound was 2600 counts per minute per mg.; conversion to the acetate yielded a product (m.p. 139–139.5° corrected) with a specific activity of 2618, and a mother liquor residue (noncrystalline) with a specific activity of 1868 corrected to the molecular weight of the free alcohol. The crystalline compound as isolated was considered radiochemically pure; the mother liquor residue did not contain material of higher radioactivity content but obviously contained solvent or other residues which prevented its crystallization and reduced its specific activity.

DISCUSSION

Biochemical considerations

The results described in the text and summarized in Table 1 show clearly that this tumor tissue could actively transform progesterone into 17-hydroxyprogesterone, androstenedione and notably testosterone, in accord with the scheme of biosynthesis of androgens demonstrated for normal testis tissue (10, 17, 18) and for virilizing interstitial-cell tumor of the

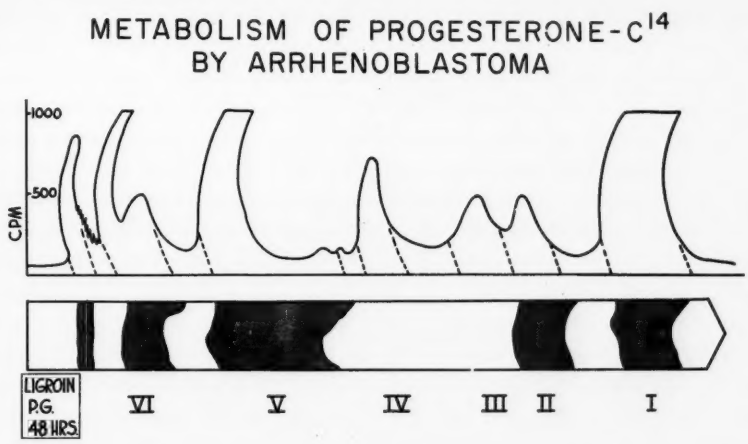

FIG. 2. Schematic reproduction of chromatogram of steroid products from incubation of progesterone-4-C^{14}. Solvent flow from left to right. Upper graph shows the peaks in radioactivity; the lower graph shows the location of the steroid zones as shaded areas. *Key:* Zone I, progesterone; Zone II, 4-androstene-3,17-dione; Zone III, 20β-hydroxy-4-pregnen-3-one; Zone IV, 20α-hydroxy-4-pregnen-3-one; Zone V, 17-hydroxyprogesterone and testosterone; Zone VI, 11β-hydroxy-4-androstene-3,17-dione.

testis (19). This mechanism has also been shown to be the pathway for the formation of androstenedione (but not testosterone) in the bovine ovary (20), in human fetal adrenal tissue (21), and in an arrhenoblastoma (22)—the latter reported since the completion of this work. It would therefore appear from the present study that the formation of androgens by arrhenoblastoma tissue follows the biosynthetic course established for normal gonadal tissues. These results are also in agreement with the recent isolation of androstenedione and 17-hydroxyprogesterone from normal ovarian tissue (4), and of testosterone from arrhenoblastoma (23).

The transformation of progesterone into the two epimeric progestational compounds, 20α- 20β-hydroxy-4-pregnen-3-one, by this tissue (Table 1) is in agreement with the occurrence of these epimers in normal ovarian and other female gonadal tissues (24). The formation from progesterone of the single 20β-hydroxy epimer by bovine corpora lutea (25) and arrhenoblastoma tissue (22) has been previously observed; both epimers are formed *in vivo* in the human (24). The significance of these two compounds, other than their postulated role as an intermediate in androgen biosynthesis (17), is obscure; it is perhaps noteworthy that neither compound is formed from progesterone by testis interstitial-cell tumor tissue (26).

No radioactive estrogen was detected in this study and in that of others (22), suggesting that estrogens are not formed by this tumor. Our results

may, however, be due to the addition in the *in vitro* experiment of relatively large quantities of carrier estrogens (Table 1), thereby reducing isotope concentration to levels below detection. The early clinical evidence of estrinization shown by our patient may have been due to estrogen formation by the tumor; estrogen may also have been formed peripherally from the testosterone elaborated by the tumor (27, 28), or again it may have been produced in the remaining ovarian tissue (see subsequent discussion).

The failure to demonstrate the formation of 11β-hydroxyandrostene-3,17-dione in this study suggests an important difference between arrhenoblastoma tissue and virilizing interstitial-cell tumor tissue of the testis. The formation of this compound and its secretion by a virilizing tumor of the testis (26) implies the presence in the latter of an enzyme, 11β-hydroxylase, which is generally associated with adrenocortical tissue, and is absent from normal testis tissue (13) and apparently also from arrhenoblastoma tissue.

The presence of testosterone in arrhenoblastoma tissue (23) and the demonstration in this study of its formation from progesterone by this type of tumor are factors considered to be quite significant in explaining the high degree of masculinization seen in these cases (5, 7–9). Normal ovarian tissue has not been shown to contain or elaborate testosterone, although the isolation of androstenedione has been reported (4). The latter androgen, although produced in significant quantity by the testis (13, 29), exhibits little androgenic activity *in the human*; the intramuscular administration of a weekly dose of 1800 mg. (300 mg. per day for six of seven days) for three or four months to 10 women with breast cancer "caused mild facial hair growth in two patients and a slight voice change in a third" (G. Escher and R. Kaufmann, private communication). Testosterone, on the other hand, produces a similar degree of virilism in women at the level of 50–100 mg. per week or less, indicating that androstenedione is only 3 to 6 per cent as active as testosterone in the human (30). Clinical virilization due to excessive formation of androstenedione might therefore be associated with a high urinary titer of 17-ketosteroids, whereas the same degree of virilism resulting from the more androgenic testosterone might be associated with only slightly elevated or even normal urinary levels of 17-ketosteroids. This would appear to be the explanation for the virilism due to arrhenoblastoma. In the cases in which urinary 17-ketosteroids are mildly elevated (9, 30), the increase is due principally to increases in the levels of androsterone and etiocholanolone (31–33), both of which are metabolites of the two androgens in question.

The histogenesis of arrhenoblastoma has not as yet been fully elucidated. These tumors consist of adenomatous cords, columns or tubules, among which are interspersed cells resembling interstitial or Leydig cells. It is presumed that the androgens are produced by the latter cells. It is note-

worthy, however, that the tumor studied here did not exhibit typical interstitial cells. In general, the intensity of virilization has been proportional to the degree of cytologic differentiation, and whenever a more mature cellular structure has been noted there have been fewer androgenic manifestations (6–9). Although often the contralateral ovary is atrophic, it is not uncommon (as in the present instance) to find evidence of follicular activity. It seems likely that this would vary with the degree and duration of the androgenic activity of the tumor. In any event, following removal of the tumor, prompt return of regular ovulatory menses is the rule.

It must be presumed that in the cases in which virilization is minimal, either androstenedione alone is elaborated or the number of androgen secreting cells are too few to secrete sufficient amounts of testosterone to produce clinical manifestations. With the evidence pointing to the latter as the androgenic principle in this clinical picture, we are at a loss to explain the failure of the Salt Lake City group to demonstrate the *in vitro* formation of testosterone from progesterone (22); it may, however, be due simply to their use of smaller amounts of radioactive precursor in their incubation studies.

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ENDEMIC GOITER IN THE UELE REGION (REPUBLIC OF CONGO). I. GENERAL ASPECTS AND FUNCTIONAL STUDIES*

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ABSTRACT

Preliminary investigations were carried out in a widespread endemic goiter area (Uele) in the north of the former Belgian Congo. The natives were usually in poor nutritional condition. The severe endemicity was demonstrated not only by the high incidence of goiter, but also by the frequency of associated syndromes such as dwarfism, mental deficiency and cretinism. Hypothyroidism was fairly common, but no case of toxic goiter was observed. The thyroid function of 166 goitrous and nongoitrous subjects was studied by means of I^{127} and I^{131} determinations. The average thyroidal I^{131} uptake was 75.3 per cent of the dose after twenty-four hours, and the thyroidal clearance rate was 43.83 ml per minute. The mean value for serum I^{127} concentration in untreated goitrous patients was in the low-normal range (4.03 μg. per 100 ml.), and in 56 per cent of 118 patients studied the level was below 4 μg. per 100 ml. Elevated PBI^{131} values were noted mostly in untreated young adults. All the data reflected the attempt of the thyroid gland to adapt to severe iodine deficiency; there were many cases of failure to compensate. The finding of significant amounts of triiodothyronine in the blood of 5 patients was possibly another indication of thyroidal adaptation to a lack of iodine. In natives living in the endemic area, the lack of iodine in the diet was directly demonstrated by the low urinary excretion of iodine (about 18 μg. daily). The iodine content of the water from the Uele region was extremely low (less than 1 μg. per liter). The water from two endemic zones close to the Uele region had a higher content of iodine, but it was still in the low range.

THE Uele endemic goiter area is probably one of the largest in the world and has the highest incidence of goiter at the present time. Located in the northern part of the former Belgian Congo, on both sides of

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Uele river, it covers a region of about 250,000 square kilometers and is occupied by some 600,000 inhabitants (Fig. 1).

Figure 2 is a detailed map of the area we investigated. Except for some hilly parts in the south, the region is fairly flat, the altitude varying between 100 and 300 meters above sea level.

Previous reports (1–3) suggest that Uele endemic goiter developed fairly recently, probably at the beginning of this century. Most of the tribes living there at the present time arrived in the region some eighty years ago, coming from the north (Sudan). The first cases of goiter were observed by Rhodain in 1915 (1). Since then, the condition has been partly described by

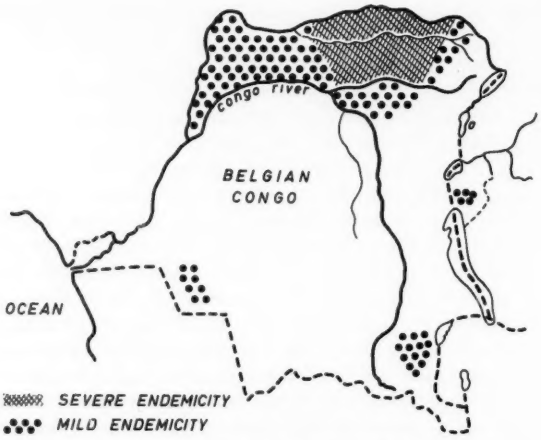

FIG. 1. General map of the former Belgian Congo.

a few authors, and some investigations have been made, mainly on the pathology of the thyroid (3–5).

We have no precise data concerning the northern borders of the endemic area (*i.e.*, beyond the frontier of the former Belgian Congo). With regard to the incidence of goiter, three zones can be distinguished (Fig. 2):

1. The marginal zone—a narrow area close to the northern bank of the Congo River—where 5 to 20 per cent of the natives are goitrous.
2. An area of average endemicity, where the incidence of goiter is between 20 and 50 per cent.
3. An area of severe endemicity (hyperendemic zone) where the incidence approaches 100 per cent.

The field of our investigations was mostly in the third area—the first time in 1958 on the west side (around Buta and Bondo), and the second time in 1959 on the east side (with Poko as center). During the first cam-

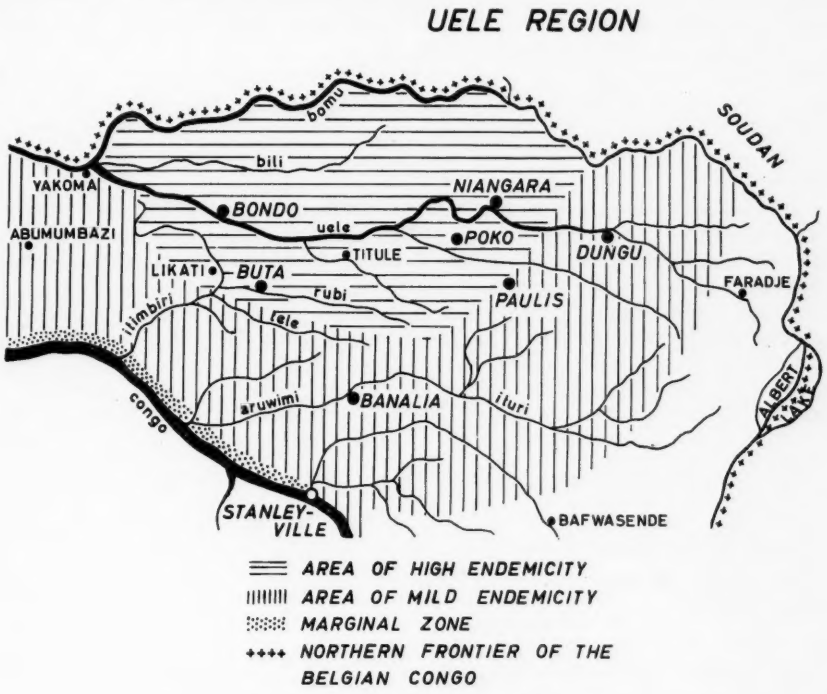

FIG. 2. Map of the endemic region.

paign, we traveled about continuously in order to get a clinical picture of the endemic. During the second campaign, we stayed in Poko most of the time, where a "field laboratory" was established close to a small country hospital in which material for functional studies could be processed for analysis.

MATERIAL AND METHODS

Subjects and procedure

Many subjects were examined in different parts of the endemic area. Our figures for the incidence of the goiter were obtained by a sampling method. For more than two years, the local medical organization (FOREAMI, or "Fondation Reine Elisabeth pour l'Assistance Médicale aux Indigènes") has carried on an extensive statistical study of the disease among the population of the Uele territories. This work is not yet completed (data will be published later). The known results are impressive; in many parts of the hyperendemic zone the prevalence of goiter approaches 100 per cent.

One hundred and sixty-six subjects from the Uele endemic area were investigated. All types of goiter were included, but most of them were of the diffusely enlarged variety. Control studies were carried out on nongoitrous patients from areas outside the Uele region and on euthyroid patients from Belgium. The general distribution of the subjects according to sex, age, and type of goiter is shown in Table 1. Functional investigations

of the thyroid included measurements of the avidity of the gland for iodine (I^{131} uptake and thyroidal clearance) and studies of the products of secretion in the blood (PBI^{127}, PBI^{131} and conversion ratio, and I^{131}-amino acids). Thyroidectomy was performed on 12 patients; results of the thyroid analysis are to be reported elsewhere (6). The samples of serum were frozen and flown in solid carbon dioxide to our laboratories in Belgium.

METHODS

Protein-bound iodine in serum (PBI). Determinations were made by means of a dry-ashing technique, according to Salter and Johnson (7) and Barker *et al.* (8). This method has been used routinely in our laboratory (Louvain) for many years; the error is within about 5 per cent.

Iodine in water. Approximately 1-liter samples were collected, and sterilized on the spot by heating three times at 90° C. In some cases, a few mg. of azide NaN_3 were

TABLE 1. DISTRIBUTION OF SUBJECTS ACCORDING TO SEX, AGE AND TYPE OF GOITER

Distribution of the Subjects

Total number	Males	Females	Adults	Schoolgirls	Cretins
166 100%	32 19.3%	134 80.7%	89 53.6%	57 34.4%	20 12.0%

Types of Goiter

Adults and schoolgirls					Cretins	
D_1*	D_2	D_3	Nodular	No goiter	$D_2 - D_3$	No goiter
44 25.5%	49 33.5%	10 6.7%	25 17%	18 12.3%	2 10%	18 90%

* D_1 = Diffuse goiter, grade 1.

added. The analysis was made essentially by the method used for PBI determination, but some modifications were needed. The sample was first carefully concentrated 100-fold by gentle boiling. In order to avoid loss of iodine during ashing, it was necessary to add some protein before placing the tubes in the oven. Human serum (pool from various blood samples) of known iodine content proved to be a convenient agent. One ml. of the water concentrate was added to the washed precipitate (Somogyi: $ZnSO_4$, 10 per cent and NaOH, 0.5 *N*) of 1 ml. of serum. Then 0.8 ml. of 4 *N* sodium carbonate was added, and the mixture dried at 80° C. for fifteen hours. Ashing was carried out at 550° C. for one and a half hours. The analysis was then made by the colorimetric procedure of Barker *et al.* (8). For each series of samples, a simultaneous determination was performed on the serum only. A number of control experiments with an I^{131}-labeled solution of known iodine content showed that recovery varied between 93 and 98 per cent by this method.

Iodine in urine. The same method was used as for iodine in water, generally without preliminary concentration. The analyses were made on 1 or 2 ml. of urine. The 24-hour urine specimens were obtained from hospitalized, carefully observed patients receiving a usual diet.

Thyroidal uptake of labeled iodine was determined by conventional methods using a Tracerlab Ratemeter with a scintillation counter P-20 C, medical type. Carrier-free

radioactive iodine was employed.[1] The counter was placed over the center of the gland, at a distance of 50 cm. from the skin. The experimentally calculated correction factor for back-scattering was 0.78. In a few cases, a lead filter was used. Results are expressed as percentages of the dose.

Thyroidal clearance was determined by the methods of Berson *et al.* (9).

Serum PBI^{131} *and the conversion ratio* were determined by the classic methods (10), using trichloroacetic acid as the precipitating agent. The samples were measured in a well-type scintillation counter and the results were calculated in percentages of the dose of I^{131} per liter of plasma. Chromatographic studies were performed on the plasma after the acidified serum was subjected to extraction with butanol in the presence of thiosulfate. The chromatographic analyses were run routinely in butanol-glacial acetic acid-water (78:5:17, v/v); butanol-dioxane (80:20, v/v) in ammonia 2 *N*; butanol-ethanol-ammonia 2 *N* (5:1:2, v/v); and butanol-isoamyl alcohol-ammonia 6 *N* (1:1:2, v/v). Spots were detected by adding known amounts of sodium iodide, 3-monoiodotyrosine, 3,5-diiodotyrosine, thyroxine and 3,5,3′-triiodothyronine, and spraying the dried strips with diazotized sulfanilic acid for the iodinated amino acids and with palladium chloride for iodide. Autoradiographs of unsprayed strips were also made, using non-screen x-ray films.

RESULTS

Clinical aspects of Uele endemic goiter

Marginal zone. In the natives of this area, the only observable sign was a diffusely enlarged thyroid gland in 15 to 20 per cent, mostly adults. On the whole, the population appeared to be healthy, and there were no complications.

Area of average endemicity. In this area, 25 per cent of the school-children had a palpable thyroid gland. Nearly half the adults were goitrous (80 per cent of these were females). As in other endemic regions, pregnancy had a definite stimulating effect on the rate of growth of the gland. All types of goiter were evident—from simple hyperplasia to large protruding multinodular masses. With time, some goiters became intrathoracic (compression of the sterno-cleido-mastoid muscles during work). Local signs of compression were frequently observed. All these patients appear to be euthyroid.

Hyperendemic area. The people of this zone were extremely primitive and poor, living in small villages along the roads or the rivers or, less frequently, in the forest. They cultivated small patches of land, kept a few goats or hens, and did some hunting or fishing. They did not give the impression of being active. The usual diet included manioc, bananas, rice and peanut oil. The supply of animal protein was extremely low (probably no more than a few hundred grams per month). In the preparation of the meals, two kinds of salts were used—one made by ashing plants, and the other obtained from salt marshes in Angola. Various samples were analyzed; their iodine content was very low or negligible.

With the exception of the children living in missionary centers or board-

[1] From the Centre d'Etudes Nucléaires of Mol, Belgium.

ing schools and the workmen on large plantations, the nutritional condition of the natives was usually poor. Malaria was frequently observed, although it was not an endemic problem. Ten per cent of the population were leprous, and under treatment with sulfone drugs. Intestinal parasitism was very common. Most of the natives had skin lesions of various kind, mainly on the legs and feet, and consequently had inguinal adenopathies. Routine examination frequently revealed enlargement of the liver and spleen, with hepatic fibrosis or cirrhosis, malaria and parasitism.

The prevalence of goiter in this area varied from 50 to 100 per cent. All types and sizes of thyroid tumors were found. Goiter appeared at an early age, and was occasionally observed in newborns and infants. Eighty per cent of the school children were goitrous. In many cases the thyroid enlargement become nodular before puberty; this is typical of zones where there is severe endemicity.

Moderate hypothyroidism was often suspected on clinical grounds. Typical hypothyroidism could be recognized in about 10 per cent of the goitrous patients—sometimes associated with dwarfism (Fig. 3).

Cases of toxic goiter were not seen. Carcinomatous degeneration of nodular goiter has been described (5), but this was exceptional. Acute suppurative thyroiditis sometimes occurred after application of some local therapy by the natives (such as pinning thorns into the skin of the neck). Cretinism was evident everywhere in this area mainly in a few villages far away from the centers. Often there was more than one case in a family. All degrees of mental deficiency existed. Investigations on cretins will be reported in a subsequent paper.

Serum protein-bound I^{127} (Fig. 4)

The determinations were carried out in 118 untreated goitrous patients. The mean value was 4.03 μg. per 100 ml. ±0.23 μg. (S.E.). Attention is called to the large number of low values—56 per cent below 4 μg., 36 per cent below 3 μg., 18 per cent below 2 μg., and 4 per cent below 1 μg. In treated goitrous patients (7 cases) from the endemic area, the PBI level was 7.31 ±0.72 μg. per 100 ml. In euthyroid Belgian controls (102 cases), the mean value was 6.50 ±0.19 μg. per 100 ml.

Thyroidal uptake of I^{131}

The uptake was determined at two, six, twenty-four, and in some cases as long as one hundred hours after giving the tracer dose of I^{131}. The results in various groups of subjects are shown in Figure 5. Detailed curves correlated with the serum PBI^{127} levels are shown in Figure 6. In treated patients the uptake was not as high as in untreated patients, but remained within high-normal limits, especially during the first few hours.

FIG. 3. Goitrous patient with hypothyroidism and dwarfism.

Thyroidal clearance of I^{131}

The clearance rate was measured in 17 cases. Although the results were not as high as expected, the mean value (43.83 ml. per minute) was significantly different ($P < 0.001$) from the mean value for the controls in Belgium (24.41 ml. per minute).

Serum PBI^{131} (conversion ratio) and other serum I^{131}-compounds (Table 2)

Results in the untreated goitrous subjects could be classified into two groups: 1) PBI^{131} level within the normal range (0.01–0.20 per cent of the dose per liter), and 2) PBI^{131} level in the hyperthyroid range. In the iodine-treated patients, results were within normal limits. In 9 patients, chromatographic studies were made of the radioiodinated compounds in the blood. Using isoamyl alcohol-ammonia as the solvent system, thyroxine represented 50 to 99 per cent of the radioactivity of the thyronines on the strip. In 5 cases, triiodothyronine was found in significant amounts (6 to 15 per cent of the radioactivity of the thyronines on the strip). The pos-

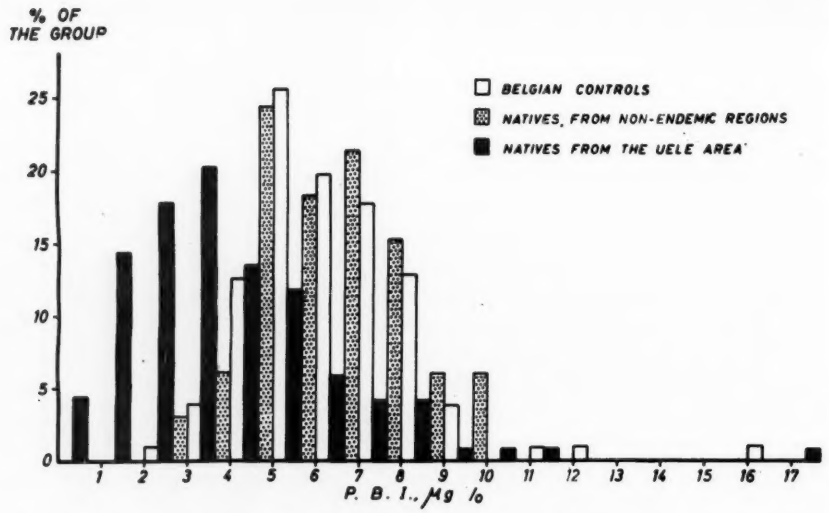

FIG. 4. Frequency distribution of serum PBI[127] values in goitrous patients, as compared with those in controls outside of the endemic area.

sibility of the coexistence of acetate derivatives of thyroxine migrating with 3,5,3′-triiodothyronine in our solvents can not be excluded.

Epidemiologic investigations

Lack of iodine in the environment is usually regarded as the most important factor in endemic goiter areas. The role of this factor had to be demonstrated in the Uele region.

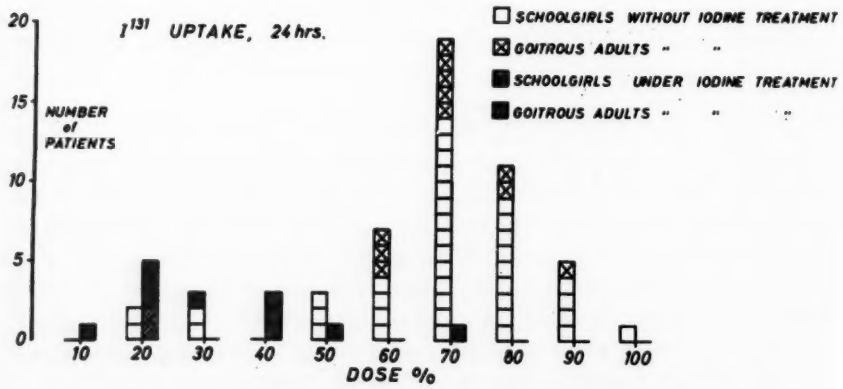

FIG. 5. Thyroidal 24-hour uptakes of I^{131} in certain groups of subjects.

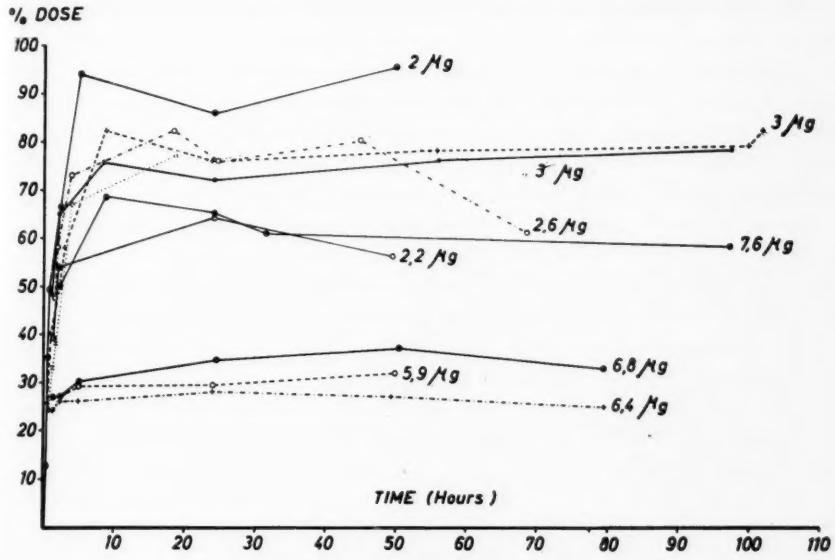

FIG. 6. Serum concentration of PBI^{127} and the thyroidal uptake of I^{131} in goitrous subjects (Poko).

TABLE 2. VARIOUS PARAMETERS OF THYROID FUNCTION

Groups	Subject No.	Age (yrs.)	Max. thyroidal uptake of I^{131} (% dose)	Conversion ratio	Serum PBI^{131} (% dose per liter)		BEI^{131}* / PBI^{131} ratio
					24 hrs.	48 hrs.	
Untreated patients	001	22	—	95	1.5	—	0.77
PBI^{131} 0.50% dose/L.	002	22	6	88	2.7	—	0.70
	014	35	78	85	1.10	—	0.69
	028	14	67	70	1.03	—	—
	015	25	95	82	0.84	1.30	—
	041	30	64	76	2.25	3.33	0.71
	017	25	79	85	1.34	—	0.67
	147	28	85	91	0.58	0.96	—
	072	20	74	93	3.40	—	—
	148	25	83	91	0.53	0.67	—
PBI^{131}* 0.09% dose/L.	018	45	69	36	0.03	0.08	—
	027	35	78	25	0.02	—	0.97
	024	30	82	35	0.07	—	—
	019	35	82	—	—	0.08	0.69
	148	42	82	56	0.05	0.05	—
	149	30	86	95	0.05	0.09	—
Iodine-treated patients	020	35	35	37	0.16	—	—
	040	34	30	4	0.03	0.05	—
	042	26	28	6	0.04	0.05	—

* Butanol-extractable iodine.

Fifty-nine samples of drinking water, collected from the three zones, were analyzed for iodine. The results are summarized in Table 3.

The iodine content was extremely low, no individual value being higher than 1 μg. per liter. Similar results were obtained in water from the main rivers in the hyperendemic area. Control studies were carried out in the non-endemic regions; 18 samples of drinking water were collected. The iodine values, although low, were significantly higher than in the endemic zone; in all but 1 sample the iodine content was more than 1.5 μg. per liter.

TABLE 3. IODINE CONTENT OF DRINKING WATER IN THE ENDEMIC ZONE

Areas	Endemicity (% population with goiter)	No. of samples	Iodine (μg./liter) (mean values)
Stanleyville-Banalia	5 to 20	4	0.62
Weko-Yaliboto	20 to 50	14	0.34
Banalia-Buta	20 to 50	8	0.35
Bondo-Monga	20 to 50	4	0.36
Monga-Yakoma	20 to 50	7	0.37
Buta-Titulé	50 to 100	3	0.25
Titulé-Bambesa	50 to 100	6	0.16
Buta-Bondo	50 to 100	8	0.15
Bondo-Likita-Sambili	50 to 100	4	0.28
Monga-Bengassou	50 to 100	5	0.19

The iodine in the diet was also investigated by determining the daily urinary iodine excretion. Urine was collected in 24-hour samples from 14 goitrous natives. The average 24-hour excretion was 18.67 μg. ±6.9 μg. ($\sigma = 25.8$). In 4 cases, iodine could not be detected in the urine. A somewhat higher mean value (23.64 μg.) was noted in 5 patients suspected of having received some kind of iodine therapy during the year 1959. Using the same method for controls in Belgium (Louvain), the urinary excretion of iodine was found to vary between 50 and 500 μg. per twenty-four hours.

DISCUSSION

From the data reported here, it may be concluded that the main epidemiologic factor in Uele goiter is a severe iodine deficiency in the diet. The iodine content of water is extremely low throughout the whole endemic area. A close parallelism is known to exist between the amounts of iodine in water and food. The dietary deficiency was directly demonstrated by the low urinary excretion of iodine. Thus, if we assume that these patients were in equilibrium with their environment with respect to dietary iodine, then the daily excretion of this element should serve as an index of the quantity of iodine ingested. By comparing the figures obtained in this

investigation with those recently reported in the literature (11, 12), it is evident that the diet of the Uele natives is deficient in iodine.

The cause of the lack of iodine is probably to be found in the nature of the soils and rocks. The agricultural institute INEAC (Yangambi, near Stanleyville) has drawn a pedologic map of this province. There are significant differences in the composition of the soils between the hyperendemic zone and the surrounding areas. It is, however, difficult to prove that the iodine lack is related to these differences.

Since the investigations of Taylor (13), hardness of water has sometimes been regarded as another epidemiologic factor. In view of this possibility, the Hydrological Department of the INEAC[2] analyzed water samples from various endemic and nonendemic regions. The calcium content was usually found to be higher in the rivers of the Uele area than in those of the center of the former Belgian Congo.

In order to study the first steps of the thyroid reaction to iodine deficiency, the subjects selected were chiefly those with diffuse enlargement of the thyroid gland.

As a result of the iodine deficiency in the Uele endemic goiter area, the thyroidal uptake of I^{131} is high. In the present study, at two hours or even earlier (Fig. 6) after ingestion of a tracer dose, the uptake values were markedly elevated. High values for thyroidal uptake have also been found by other investigators, *e.g.*, Stanbury *et al.* (14) and Roche *et al.* (11), even without significant enlargement of the thyroid (15). In our study the individual results did not indicate any significant difference in the avidity of the thyroid for radioiodine between goitrous and nongoitrous patients living in a high endemic zone. Roche made similar observations in Venezuela (11, 15).

The thyroidal clearance rate in our patients was also significantly elevated as compared with the controls. For some unknown reason, the elevation was not as high as expected on the basis of the uptake data.

As a result of the lack of iodine, the PBI^{127} levels were low, and the difference between the untreated and the iodine-treated goitrous patients was significant ($P < 0.001$). It should be stressed that the mean value was 4.03 μg. per 100 ml. in 118 untreated goitrous patients. In this group, 56 per cent of the values were lower than 4 μg. per 100 ml. In natives outside the endemic area (controls), only 9.1 per cent of the values were below 4 μg., and among 120 euthyroid controls living in Belgium only 4.9 per cent of the values were below this level. These concentrations of PBI are much lower than those usually reported for other regions (12, 14, 16), except for Guatemala (17). The PBI levels we found in cretins are also

[2] Institut National pour l'Etude Agronomique du Congo Belge.

lower than those reported by Raman and Beierwaltes (18). This fact stresses again the importance of the iodine deficiency in the Uele. The thyroid attempts to adapt itself to the deficiency, but in many cases it fails to produce sufficient amounts of hormone.

In many of the subjects in our study, the serum PBI^{131} level was in the "hyperthyroid range." High values were found mostly in young adults. Studies are in progress in order to determine whether there is a true correlation between the age of the subject and the level of PBI^{131}. On the other hand, differences in the iodine pool could account for the two different types of response. It is not surprising to find a high concentration of PBI^{131} in patients living in iodine-deficient regions. A small amount of iodine offered to the gland will be bound more rapidly to protein, and several studies have demonstrated the high turnover of iodine in such subjects. In iodine-treated patients the PBI^{131} levels are not elevated.

Of particular interest is the finding of 3,5,3′-triiodothyronine in the blood of some subjects. In euthyroid patients from a nonendemic zone, it is difficult to detect significant amounts of triiodothyronine. In hyperthyroid patients, triiodothyronine is more frequently found (19, 20). In euthyroid subjects stimulated by the administration of thyrotropin, there is an elevated level of tagged triiodothyronine (20, 21). Lachiver and Leloup (22), Querido *et al.* (23) and Bois and Larsson (24), in experiments with rats, found that longstanding iodine deficiency results in an increased concentration of triiodothyronine in the thyroid. If, as may be expected, goitrous patients from an iodine-deficient area are under a high degree of stimulation by thyrotropin, this would explain the significant amount of triiodothyronine we found. Of the thyroid hormones, triiodothyronine has the highest activity per atom of iodine. Its enhanced formation has the character of an adaptive reaction to iodine deficiency. This would explain the discrepancy between the clinical picture and the serum PBI level in some cases.

Acetate derivatives are known to run close to 3,5,3′-triiodothyronine in the chromatogram, with the solvents we used. The possibility of their presence was not investigated here, but new studies are in progress in order to check this important point.

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QUANTITATIVE OBSERVATIONS WITH THE TRIIODOTHYRONINE SUPPRESSION TEST OF THYROID FUNCTION*

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ABSTRACT

Triiodothyronine (T_3), 120 to 150 μg. per day, was administered for seven days to 50 thyrotoxic patients and 109 euthyroid subjects. The thyroidal I^{131} uptake rate was determined before and during administration of T_3. Diagnostic borderlines were determined by analysis of the ratios of these uptake rates. The triiodothyronine suppression test is a useful diagnostic aid, and is the suppression test of choice. Diagnostic data are obtained sooner and are less likely to be affected by prior antithyroid medication than with the thyroxine suppression test.

INTRODUCTION

THE uptake of radioactive iodine by the thyroid gland of thyrotoxic patients cannot be lowered by the administration of thyroid hormone. In euthyroid subjects, however, the uptake can be considerably reduced, and with a sufficiently large dose of hormone the uptake of I^{131} should fall into the hypothyroid range (1). In this event diagnostic differentiation would be simple, but in practice the dose of hormone required for complete suppression would have to be individually determined for each patient.

Usually the effect on thyroidal I^{131} uptake of the administration of a fixed dose of thyroid hormone for a fixed time is assessed in relation to the expected response. This assessment is dependent upon previous experience with the selected dose and the duration of administration of the particular hormone. Dried thyroid, thyroxine (T_4) and triiodothyronine (T_3) have each been used as suppressing agents (2–4). Since thyroxine and triiodothyronine are pure substances, the response to their administration should be more uniform than the response to a biologic preparation such as dried thyroid.

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The use of thyroxine as a suppressing agent (5) has been previously investigated in our unit. In the present study, the effect of triiodothyronine on the thyroidal I^{131} uptake rate was determined, and the two tests were compared.

METHOD

Each patient was given an intravenous injection of 10 μc. of I^{131}, and the thyroidal uptake and renal excretion rates were calculated by methods previously described (6). The results of each test were expressed as k_1 (thyroidal) and k_2 (renal) rates, these being the fraction of iodide removed each minute from the extrathyroidal iodide space.

Patients were included in this study only when the final diagnosis was clearly established. The diagnosis was made on the basis of an initial I^{131} uptake rate that was clearly within either the euthyroid or the hyperthyroid range (7), or on the basis of clinical assessment and evaluation of the response to therapy or the course of the patient's disease.

After an initial uptake test, the patient was given 40–50 μg. of *l*-triiodothyronine[1] every eight hours for seven days. The I^{131} uptake test was repeated on the seventh day, not more than three hours after the last dose.

RESULTS

Thyrotoxic patients

Of 50 thyroxtoxic patients studied, 9 had, and 41 had not received prior medication with antithyroid drugs. Table 1 shows the thyroidal uptake rates (k_1) observed in all of these cases before and during the ingestion of triiodothyronine; in every fourth column this change is expressed as x, the logarithm of the ratio of k_1 values, where

$$x = \log_{10} \frac{k_1 \text{ before triiodothyronine}}{k_1 \text{ with triiodothyronine}} .$$

A postive value of x represents a fall in k_1 when the patient is taking triiodothyronine, and a negative value represents a rise in k_1.

The application of Chauvenet's criterion[2] (8) eliminated the 3 most negative values of x. The remaining 47 values were normally distributed, a χ^2 test of goodness of fit yielding a value $0.5 < P < .10$. The characteristics were as follows:

Mean	+0.0058
Standard deviation of a single value	±0.0635
Standard error of the mean	±0.0093

The mean did not differ significantly from zero.

[1] *l*-Triiodothyronine, Cynomel, was generously donated by Smith Kline & French (Australia) Ltd.

[2] Chauvenet's criterion was applied to ensure that the diagnostic borderlines were calculated from normally distributed data. In no instance did the rejection of cases alter the significance of the results.

When the 41 patients who had not had prior medication were assessed separately, Chauvenet's criterion again eliminated the 3 most negative values of x. The mean for the remaining 38 cases did not differ significantly from zero, nor did that for the 9 patients who had had prior medication. The determination of diagnostic borderlines was therefore made, using data from all thyrotoxic patients.

In the thyroxine series, the mean for patients who had no prior medication was significantly different from zero, whereas the mean for those who had had prior medication was not; consequently the analysis to determine diagnostic limits in patients undergoing thyroxine suppression of thyroid function was made on each of these groups separately (5).

TABLE 1. EFFECT OF TRIIODOTHYRONINE (T_3) ON k_1 OF THYROTOXIC PATIENTS

No prior medication				No prior medication				Prior antithyroid medication			
	Rate, 10^2 k_1				Rate, 10^2 k_1				Rate, 10^2 k_1		
Case No.	Initial	During admin. of T_3	x	Case No.	Initial	During admin. of T_3	x	Case No.	Initial	During admin. of T_3	x
1	14	11	0.09	22	15	15	0.00	42	37	31	0.07
2	36	29	0.09	23	33	33	0.00	43	39	35	0.04
3	12	9.8	0.08	24	13	13	0.00	44	8.4	7.7	0.04
4	21	18	0.08	25	10.7	11.0	−0.01	45	7.3	6.9	0.03
5	17	14	0.08	26	16.8	17.4	−0.01	46	21	20	0.02
6	9.9	8.3	0.08	27	12	13	−0.02	47	6.2	6.2	0.00
7	23	20	0.07	28	9.2	9.8	−0.03	48	18	20	−0.04
8	18	16	0.07	29	24	26	−0.03	49	6.7	7.9	−0.07
9	15	13	0.06	30	6.4	7.0	−0.04	50	25	35	−0.14
10	18	16	0.06	31	6.8	7.5	−0.04				
11	25	22	0.06	32	29	33	−0.05				
12	7.9	6.9	0.06	33	5.9	6.8	−0.06				
13	6.5	5.7	0.05	34	17	21	−0.09				
14	31	28	0.05	35	12	15	−0.11				
15	6.7	6.0	0.05	36	45	58	−0.11				
16	25	23	0.05	37	5.7	7.3	−0.11				
17	11	10	0.05	38	7.0	9.3	−0.12				
18	11	9.7	0.04	39	39	64	−0.21				
19	35	34	0.01	40	10	26	−0.41				
20	14	14	0.00	41	4.4	19	−0.64				
21	19	19	0.00								

Euthyroid subjects

Of the euthyroid subjects studied, many had a goiter and many had an initial thyroidal I^{131} uptake rate in the borderline euthyroid-hyperthyroid zone (7).

In 4 cases out of a total of 109, triiodothyronine in doses of 120 to 150 μg. per day for seven days lowered k_1 to zero, with a corresponding x value of ∞; for the remaining 105 subjects the range of x was from 0.069 to 1.97, with a mode of 0.75.

The distribution of x for euthyroid subjects was skew and not normal, but the lower values of x are of most interest, since they overlapped those found in thyrotoxic patients. A plot of the frequency of occurrence of the lower values (Table 2) shows that a value of x less than 0.063 would be expected in 1 per cent of euthyroid cases.

It can be shown that the function log $(1+x)$ is normally distributed. A test of goodness of fit yields $\chi_9^2=9.29$, for which $.50>P>.30$. The percentage points indicated by this curve are: 1 per cent ($x=0.105$) and $\frac{1}{2}$ per cent ($x=0.048$) compared with 1 per cent ($x=0.063$) and $\frac{1}{2}$ per cent ($x=0.014$) obtained by a plot of the lowest values. Here the latter values are used for comparison with those in our previous paper (5).

Effect of triiodothyronine on the renal rate (k_2)

Although in these studies the determination of the renal excretion rate

TABLE 2. EFFECT OF TRIIODOTHYRONINE (T_3) ON k_1 OF 6 EUTHYROID PATIENTS* IN WHOM THYROID FUNCTION WAS THE MOST DIFFICULT TO SUPPRESS

	Patient M.K.	Patient R.W.	Patient E.B.	Patient A.W.	Patient E.B.	Patient J.H.
Age (yrs.)	68	72	58	56	34	49
Goiter size and type†	1D	1D	1N	0	1D	2N
T_3, μg./day	150	150	150	120	150	150
No. of days	7	7	7	7	7	7
Rate $10^3 k_1$ Initial	9.55	11.2	6.20	8.15	15.4	5.76
Rate $10^3 k_1$ During admin. of T_3	8.18	8.70	4.62	5.56	10.0	3.49
x	.069	.109	.128	.165	.178	.218

* Six out of 109 euthyroid patients—4 females and 2 males.
† Goiter sizes were classified 0 to 4; D = diffuse; N = nodular.

(k_2) is less precise than that of k_1, it is possible to demonstrate a small rise in k_2 during administration of triiodothyronine

The expression

$$y = \log_{10} \frac{k_2 \text{ before triiodothyronine}}{k_2 \text{ with triiodothyronine}}$$

was evaluated for 142 patients of whom 45 were thyrotoxic and 97 euthyroid.

Application of Chauvenet's criterion eliminated the 5 most negative values and the largest positive value of y, leaving 136 cases with the following characteristics:

Mean	−0.0274
Standard deviation of a single value	±0.1337
Standard error of the mean	±0.0115

The mean differed significantly from zero, with $.02>P>.01$; and the mean rise in k_2 was 6.5 per cent.

DISCUSSION

Since 20 per cent of euthyroid patients and 50 per cent of hyperthyroid patients need a suppression test to confirm the diagnosis,[3] 1 per cent of those requiring suppression can be wrong if we allow 1 in 500 euthyroid cases to be wrongly diagnosed as hyperthyroid and 1 in 200 thyrotoxic cases to be wrongly diagnosed as euthyroid. A greater error is permissible in wrongly diagnosing thyrotoxic cases as euthyroid because no irreversible therapy is given and the true thyroid state will later become obvious.

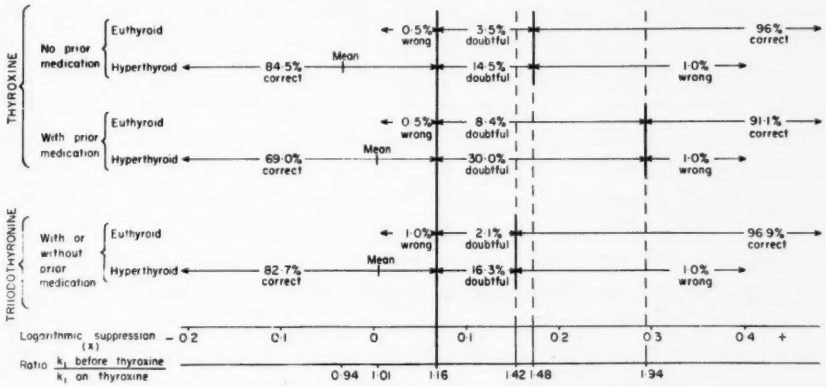

FIG. 1. Percentages of patients in various diagnostic zones, as judged by triiodothyronine and thyroxine suppression tests.

Diagnostic borderlines for the suppression ratios can be drawn as follows: if the ratio

$$\frac{k_1 \text{ before } T_3}{k_1 \text{ with } T_3}$$

is less than 1.16 ($x = .063$) the patient is thyrotoxic, and if it is greater than 1.42 ($x = .152$) the patient is euthyroid (Fig. 1). Thus 2.1 per cent of patients who are euthyroid and 16.8 per cent of those who are hyperthyroid will fall between these limits.

The rise in renal excretion rate could be due to an increase in the metabolic rate resulting from the ingested dose of triiodothyronine (9).

These results are similar to those obtained with thyroxine. If the defined ratio is less than 1.16 with either T_3 or T_4, the patient is classified as hyper-

[3] These percentages are obtained if the diagnostic limits of Oddie *et al.* (7) are applied. These limits allow not more than 1 euthyroid patient in 1000 to be wrongly classified as thyrotoxic on the basis of a single test.

thyroid. If the ratio exceeds 1.42 with T_3, or 1.48 with T_4 and no prior medication (or 1.94 with T_4 and prior medication), the patient is classified as euthyroid. A larger statistical error in wrongly diagnosing euthyroid patients as thyrotoxic is allowed in the triiodothyronine series, although the log $(1+x)$ analysis would indicate that the error is actually less than 1 per cent. The triiodothyronine series is smaller than the thyroxine series and the percentages quoted are those obtained from fitted statistical distributions. None of the triiodothyronine suppression values in euthyroid subjects fell in the thyrotoxic range.

The percentage of cases falling into the doubtful range is similar—T_4 with no prior medication (euthyroid 3.5 per cent and hyperthyroid 14.5 per cent); T_4 with prior medication (euthyroid 8.4 per cent and hyperthyroid 30.0 per cent); T_3 (euthyroid 2.1 per cent and hyperthyroid 16.3 per cent). Prior medication with antithyroid drugs does not demonstrably affect the triiodothyronine suppression test, whereas it definitely affects the thyroxine suppression test. Fewer patients who had had prior medication were seen during the triiodothyronine study, probably because fewer patients are now being treated before referral for assessment. Thyroxine was administered for three weeks, and T_3 for only one week. Therefore, if there is a real difference between the effects of T_3 and T_4, it is possible that the failure to demonstrate a reduction in I^{131} uptake in thyrotoxic patients during administration of T_3 following prior medication with antithyroid drugs is related to the relative duration of the tests. Hyperplasia in the presence of thyrotoxicosis may require time to disappear.

Attempts to shorten the duration of triiodothyronine administration have been unsatisfactory. One patient was given 150 μg. of triiodothyronine every hour for six hours, but in spite of marked tachycardia and sweating no depression in I^{131} uptake was demonstrated, although routine testing over a period of one week showed euthyroid suppression. Administration of 150 μg. of triiodothyronine every eight hours for four days failed to demonstrate suppression in a proportion of euthyroid patients who later showed euthyroid suppression after administration of the drug for seven days. It is therefore necessary to allow sufficient time for suppression to develop before attempting to assess the results of the test.

It would appear that the triiodothyronine suppression test is almost as reliable as the thyroxine test, and has two advantages: 1) it is less likely to be affected by prior medication, and 2) it takes only one week instead of three weeks to arrive at a diagnosis.

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A CASE OF PRIMARY HYPERPARATHYROIDISM WITH SPONTANEOUS REMISSION FOLLOWING INFARCTION OF THE ADENOMA WITH DEVELOPMENT OF HYPOCALCEMIC TETANY

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ABSTRACT

A case of spontaneous infarction of a parathyroid adenoma is reported. The adenoma had been hyperactive, as demonstrated by the extensive bony lesions on x-ray examination, but the patient had been asymptomatic. Following the infarction, symptoms of hypocalcemic tetany developed. The clinical impression was confirmed at the time of surgical removal of the infarcted adenoma.

SPONTANEOUS infarction of a parathyroid adenoma is an unusual entity. To our knowledge, only 3 cases have been previously reported (1–3). In a patient recently observed the clinical course was sufficiently different to warrant presentation.

CASE REPORT

R. H., a 19-year-old white male was referred to the University Hospital with unexplained symptoms of tetany. He had been in good health until six weeks prior to admission, when aching pain had developed in the inguinal areas. This pain persisted intermittently for one to two weeks, increasing in intensity on exertion. It was accompanied by generalized weakness, which continued until the time of admission. Episodes of tingling of the extremities followed cessation of the pain, and twitching of the facial muscles while shaving became apparent. With the onset of these symptoms, pain and localized tenderness developed in the left anterior neck, persisting for several days and then regressing. Symptoms of tetany increased in frequency and the patient was admitted to a local hospital, where the concentration of serum calcium was found to be 5.1 mg. per 100 ml. and serum phosphorus 3.5 mg. per 100 ml. The alkaline phosphatase level was 50.7 King-Armstrong units, and the blood urea nitrogen level 5.2 mg. per 100 ml. The specific gravity of the urine was 1.010 and 1.026. The patient was then referred to the University Hospital for evaluation.

The previous medical history included an accidentally fractured maxilla three years before, and an injury to the left arm resulting in the removal of a "calcium deposit" from the elbow six months before admission. There was no other history of fracture or bone pain. He had not had abdominal pain, nausea, vomiting, constipation, polydypsia, polyuria, nocturia, or renal colic.

On admission his blood pressure was 130/80 mm. Hg. Trousseau's sign appeared two

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minutes after the cuff was inflated past systolic pressure, and Chvostek's sign was also present. Examination of the eyes, both routinely and with the slit-lamp, did not show any calcification. A nodule measuring 1 cm. by 2 cm. was palpable in the left lobe of the thyroid. The remainder of the examination revealed nothing abnormal.

Initial laboratory values: Hemoglobin level 13.4 Gm. per 100 ml., hematocrit 41 mm., white blood cell count 6,300 per cu. mm., and differential count and blood morphology normal. The urine had a specific gravity of 1.005 and contained no albumin; the sediment was normal. The serum concentration of sodium was 136 mEq., potassium 3.7 mEq., chloride 104.5 mEq., and carbon dioxide 26.1 mEq. per liter; the blood urea nitrogen level was 7.0 mg., serum calcium 6.9 mg., and phosphorus 3.8 mg. per 100 ml. Alkaline phosphatase activity was 8.6 Bessy-Lowry units. An electrocardiogram was within normal limits except for a QT interval of 0.40 second. An electroencephalogram was normal. A phenolsulfonphthalein test showed 38 per cent excretion in fifteen minutes with a total two-hour excretion of 60 per cent.

Tubular reabsorption of phosphorus was measured; on one occasion there was 98 per cent reabsorption and on another, 91 per cent. A calcium infusion test was performed, in which the patient received 1050 mg. of calcium in 1000 ml. of isotonic sodium chloride over a four-hour period. The serum calcium concentration rose from an initial value of 7.0 mg. per 100 ml. to a maximum of 8.3 mg. per 100 ml. during the infusion, and then returned to the basal level. Serum phosphorus concentration rose from an initial value of 3.7 mg. per 100 ml. to a maximum of 4.8 mg. per 100 ml. eight hours after the beginning of the infusion and then returned to the initial level. Urinary phosphorus excretion was depressed during the day of the infusion, and increased during the next 48-hour period. These results seemed to indicate the presence of some functional parathyroid tissue. Urinary titratable acidity was measured during a three-day period of oral ammonium chloride administration, and the patient demonstrated ability to excrete an acid load.

Numerous cystic lesions were noted in bone roentgenograms. Figure 1 shows the presence of these lesions in the bony pelvis and each femur, as well as calcification in the region of the kidneys. Figure 2 shows a large cystic lesion in the left humerus. Figure 3 (left) shows subperiosteal bone resorption in the phalanges. Other films, not shown here, revealed scattered cystic lesions in the ribs, loss of the lamina dura, and demineralization of the skull.

The x-ray findings were considered diagnostic of hyperparathyroidism, although the patient had presented with hypocalcemic tetany. It was thought that this apparent dichotomy could best be explained by postulating the previous existence of a parathyroid adenoma which had ceased to function, probably because of infarction. In view of the palpable nodule in the left thyroid area, surgical exploration was considered warranted. The patient was treated with dihydrotachysterol until the serum calcium concentration reached a normal level.

At operation a nodule measuring 3.5 cm. by 2.5 cm. was found posterior and lateral to the left lobe of the thyroid. Areas of necrosis were evident throughout its substance. The diagnosis was confirmed by the pathologic picture, as shown in the photomicrograph in Figure 4. The capsule surrounding the adenoma was intact and the central area was necrotic, with a rim of viable "Wasserhelle" cells around the periphery.

The patient recovered uneventfully from the operation and was maintained for a short period of time with dihydrotachysterol and calcium lactate. At the time of discharge his serum calcium level was 9.9 mg. per 100 ml., and he was instructed to continue the calcium as his only medication.

He was seen again two months after operation. He was essentially asymptomatic ex-

FIG. 1. Cystic lesions of the bony pelvis, and renal calcifications are indicated by the arrows.

FIG. 2. A cystic lesion of the left humerus.

cept for some tingling of the hands when he compressed the blood supply to the arm. He had noted no muscle spasm. Chvostek's sign was present, but there was no Trousseau sign. The serum calcium level was 8.8 mg. per 100 ml. X-ray examination at that time showed no change in the cystic lesions, but the areas of subperiosteal resorption in the phalanges were being repaired (Fig. 3, right).

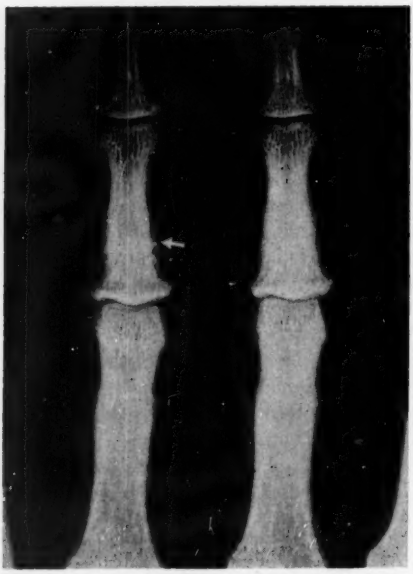

FIG. 3. On the left is a roentgenogram of one of the phalanges taken at the time of admission; note the subperiosteal bone resorption. On the right is a roentgenogram of the same area taken four weeks after discharge, showing evidence of repair.

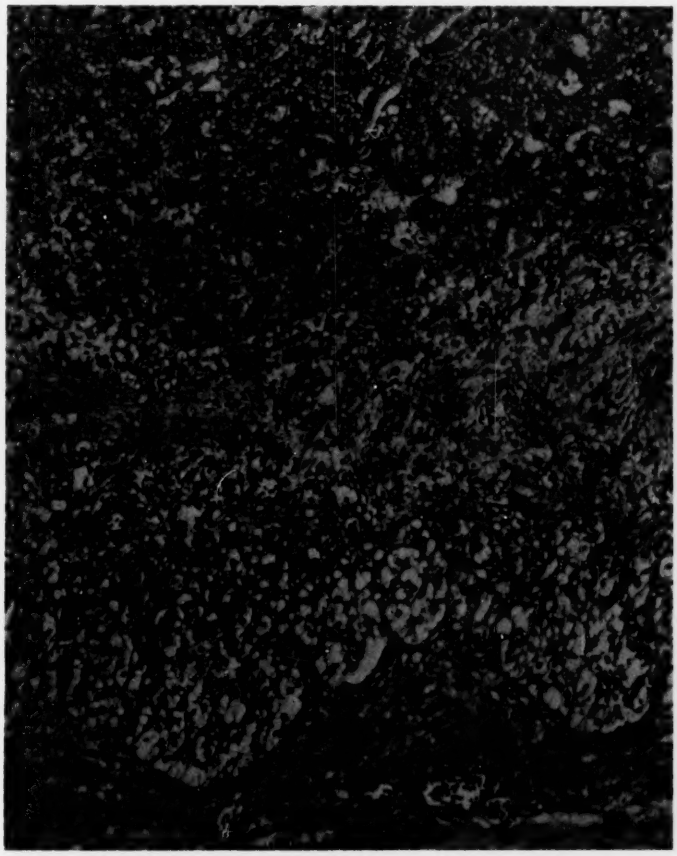

FIG. 4. Photomicrograph of the adenoma removed at operation. The necrotic central portion of the lesion is visible in the upper half of the photograph, and a thin layer of persistent tumor cells is present just above the fibrous capsule.

DISCUSSION

The patient undoubtedly had hyperparathyroidism as shown by the bony changes seen on x-ray examination, and the renal calcifications. The disease was apparently asymptomatic (as it frequently is), and when the adenoma became infarcted, definite symptoms of tetany developed. It is known that tetany following operation for parathyroid adenoma is much more common when bony lesions are present (4).

This case differs in its clinical course from the few previously reported. Dowlatabadi (1) described a fatal case of parathyroid poisoning in which a necrotic adenoma was found at autopsy. In 1946 Norris (2) reported

the case of a 48-year-old female with tetany who was found to have an infarcted parathyroid adenoma at operation; no evidence was presented to suggest that the adenoma had been hyperactive at any time. In the case reported by Howard *et al.* (3), the patient presented with symptoms of duodenal ulcer and renal insufficiency and a diagnosis of hyperparathyroidism was made. During hospitalization the adenoma became infarcted. This was confirmed by surgical exploration. The patient showed mild symptoms of hypocalcemia after the infarction.

Other examples of spontaneous remission from hyperparathyroidism have been cited in the literature (5, 6). Yet the evidence for remission in all cases was clinical (*e.g.*, regression of bony lesions on x-ray examination) and no evidence for infarction of an adenoma was presented.

The clinical course in the present patient is one which would be expected following surgical removal of a hyperactive parathyroid adenoma when the osseous system is involved. In this case, however, there was a remission of the disease when the adenoma became infarcted. The true incidence of such an entity is difficult to determine, since the primary disease could well be asymptomatic, as it was here; moreover, if the osseous system were not involved, symptoms of hypocalcemia would probably not develop.

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EFFECT OF o,p'DDD IN A PATIENT WITH CUSHING'S SYNDROME*

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ABSTRACT

A total of 546 Gm. of o,p'DDD was administered over a 16-week period to a patient with Cushing's syndrome apparently due to nontumorous hyperfunction. A rapid and significant decrease in adrenocortical function occurred in association with marked clinical improvement. Six weeks after discontinuance of the therapy the urinary excretion of neutral 17-ketosteroids increased and the drug was restarted; the urinary 17-KS level promptly returned to normal. Clinical and biochemical remission is being maintained with 2 Gm. of the drug daily, and there have been no significant toxic reactions. In view of this experience, o,p'DDD may prove to be a useful agent in the therapy of nontumorous Cushing's syndrome. It is still too early, however, to assess the long-term effects.

THE administration of DDD[1] to dogs results in extensive necrosis and atrophy of the adrenal cortex (1, 2). Systematic fractionation of the technical material and biologic evaluation of each fraction has led to the isolation (3, 4) of a strongly active, less toxic isomer known as o,p'DDD.[2] This drug causes selective adrenocortical atrophy in the dog, associated with a decrease in the rate of secretion of 17-hydroxycorticoids and in the response of the adrenal cortex to corticotropin (ACTH) (3, 5).

During the past few years, we have been engaged in a study of the effects of DDD and its derivatives in subjects with carcinoma of the breast and prostate. We found that o,p'DDD suppressed adrenocortical responsiveness, although the basal steroid excretions were generally unchanged (6).

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[1] 2,2-Bis(parachlorophenyl)-1,1-dichloroethane.

[2] 2,2-Bis(2-chlorophenyl-4-chlorophenyl)-1,1-dichloroethane. The o,p'DDD was obtained from the Burrough Brothers Manufacturing Co., Baltimore, Maryland, through the courtesy of the Endocrinology Branch of the Cancer Chemotherapy National Service Center of the National Institutes of Health.

Encouraging results have recently been reported with the use of this isomer in patients with adrenal cancer accompanied in some instances by Cushing's syndrome (7). On the basis of these observations, we extended the use of o,p'DDD to the treatment of nontumorous Cushing's syndrome. The effectiveness of the drug in the first patient so treated prompted the present report.

METHODS

Urinary 17-ketosteroids (17-KS) were determined by the method of Klendshoj *et al.* (8) (normal range for the adult female, 6–12 mg. per twenty-four hours). Levels of 17,21-dihydroxycorticoids in the urine following β-glucuronidase hydrolysis (normal range, 2–10 mg. per twenty-four hours) and in the blood (normal range, 5–20 μg. per 100 ml.) were measured by Peterson and associates' modifications of the Silber-Porter method (9). ACTH tests were performed according to the following protocol: After a control 24-hour urine collection was obtained, a dose of 25 U.S.P. units of ACTH was dissolved in 1000 ml. of 5 per cent glucose in water and administered intravenously over an eight-hour period. Blood specimens were drawn for determination of plasma 17-hydroxycorticoids (17-OH-CS) immediately before and after completion of the infusion. A second specimen of urine was collected for twenty-four hours, including the period of infusion, and analyzed for steroids. After 1959, blood glucose determinations were performed according to the glucose oxidase technique of Teller (10) (normal fasting value, 65–105 mg. per 100 ml.); previously the blood glucose levels had been determined according to a modification of the method of Folin and Wu (11) (normal fasting value, 70–115 mg. per 100 ml.).

CASE REPORT

S.S., a 36-year-old white female was first seen in the Endocrine Clinic of The Jewish Hospital of Brooklyn in November 1952, with a history of oligomenorrhea and asthenia of two years' duration. The findings on physical examination were normal except for facial plethora, hirsutism, and acne over the face and trunk. The escutcheon was male in type but the clitoris was not enlarged. The 24-hour urinary excretion of the neutral 17-ketosteroids was 11.0 mg. A roentgenologic skeletal survey disclosed mild demineralization of the lumbodorsal spine and pelvis, with a normal sella turcica. Results of a glucose tolerance test were within normal limits (Table 1). An intravenous pyelogram and perirenal gas insufflation failed to reveal any gross adrenal abnormalities.

Between the years 1953 and 1956 the patient experienced increasing fatigability, muscle weakness and the development of progressive obesity limited to the face, neck and trunk. Scattered ecchymotic and purpuric areas appeared on the skin and numerous purplish abdominal striae became visible. In June 1956 the patient was admitted to the hospital for evaluation. Roentgenography revealed progression of the previously described osteoporosis, with a normal sella turcica. The fasting blood sugar level was normal, but there was decreased glucose tolerance (Table 1). Perirenal gas insufflation still failed to demonstrate any abnormalities. The 24-hour urinary excretions of 17-OH-CS and neutral 17-KS were 15.0 and 18.7 mg. respectively. Following an eight-hour intravenous ACTH stimulation test the respective values rose to 26.7 and 89.6 mg. A cortisone suppression test over a one-week period resulted in reduction of the 24-hour urinary excretion of neutral 17-KS to 4.9 mg. The hyperactive adrenocortical response to ACTH, the positive results with the cortisone suppression test, the prolonged clinical

TABLE 1. RESULTS OF GLUCOSE TOLERANCE TESTS BEFORE AND DURING THERAPY WITH o,p'DDD

Date	Blood glucose level (mg. per 100 ml.)						Status of therapy
	Minutes after ingestion of glucose						
	0	30	60	120	180	240	
Nov. 1952	76	102	135	126	89		No treatment
June 1956	95	190	200	210	95		No treatment; just prior to irrad. of pituitary
March 1960	91	154	148	128	114	103	Sixth week of o,p'DDD therapy
May 1960	94	130	138	138	120		Sixteenth week of o,p'DDD therapy

history, and the negative adrenal x-ray findings suggested the diagnosis of nontumorous Cushing's syndrome. The patient was therefore given a course of x-ray therapy (5400 r) delivered to the pituitary over a 40-day period. Little subjective or objective improvement was experienced, however, and in January 1960 the patient was again admitted to the hospital for evaluation.

Upon physical examination (Fig. 1), the patient had a plethoric moon facies with a moderate growth of hair along the side of the jaw and chin, truncal obesity, purple striae in the flanks, and a prominent cervicodorsal fat pad. Her weight was 67 Kg., and blood pressure 150/100 mm. Hg. There was a marked acneiform eruption over the face, back and chest and scattered ecchymotic and purpuric areas on the upper and lower extremities. The pubic hair was of masculine distribution but the clitoris was not enlarged. Roentgenography again revealed considerable osteoporosis and a normal sella turcica. Visual fields were within normal limits. The 24-hour urinary excretions of 17-OH-CS and neutral 17-KS were increased to 19.6 and 27.2 mg. respectively (Table 2). Following the intravenous administration of ACTH over an eight-hour period, the 24-hour urinary excretion of neutral 17-KS rose to 76.8 mg. and the plasma 17-OH-CS level increased from 50 μg. to 90 μg. per 100 ml.

RESULTS OF TREATMENT WITH o,p'DDD

A total of 546 Gm. of o,p'DDD was administered over a 16-week period. The initial dosage was 10 Gm. daily in four fractional doses. After four weeks of therapy, severe nausea, recurrent vomiting and somnolence necessitated a decrease in the dosage to 4 Gm. and then to 3 Gm. daily (Table 2). With the latter schedule there was considerable reduction in the gastro-intestinal and central nervous system manifestations.

As may be seen in Table 2, there was a steady diminution in the urinary excretions of 17-OH-CS and 17-KS, which reached normal levels by the third and fifth weeks respectively. A small further decrease occurred in both urinary steroid fractions during the latter part of the treatment period. At no time, however, did the values decrease below normal. An eight-hour intravenous ACTH test was performed on three separate occasions (fourth, sixth and sixteenth weeks). In each instance adreno-

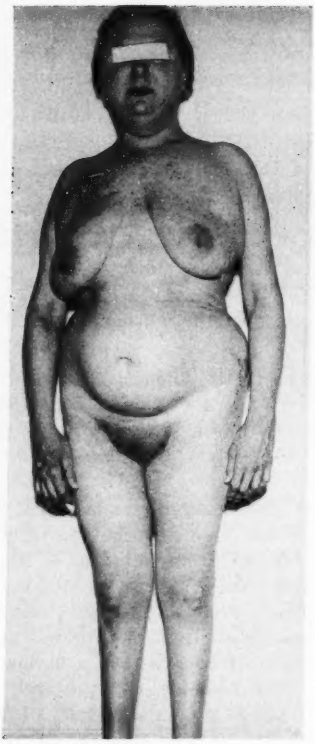

FIG. 1. The patient prior to therapy, illustrating the typical features of Cushing's syndrome.

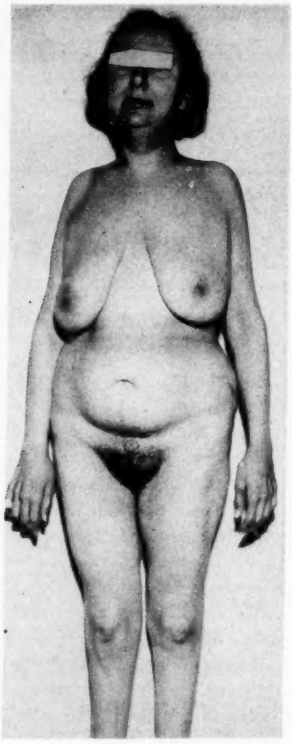

FIG. 2. The patient after sixteen weeks of o,p'DDD therapy, showing the virtual disappearance of the clinical manifestations of Cushing's syndrome.

cortical responsiveness was found to be suppressed. The most marked suppression occurred during the period of highest dosage (Table 2).

The clinical course was marked by progressive improvement. Objective clinical evidence of a response was noticeable by the fifth week of therapy and continued steadily (Fig. 2). The facial edema and plethoric appearance subsided, and there was a pronounced diminution in the facial and body acne. Little change occurred in facial hirsutism or the growth of cephalic hair. The purplish abdominal striae lightened, muscle power improved and the purpuric lesions disappeared. Menstrual bleeding began about the middle of the treatment period and recurred more or less regularly thereafter. No Addisonian-like pigmentation was noted. A weight loss of 10 Kg. and a decrease in the blood pressure to 140/85 mm. Hg took place

TABLE 2. URINARY 17-KS AND 17-OH-CS EXCRETION AND PLASMA 17-OH-CS LEVEL AND THE RESPONSE TO ACTH BEFORE AND DURING ADMINISTRATION OF o,p'DDD

Time (wk.)	Dosage of o,p'DDD (Gm./24 hrs.)	Wt. of patient (Kg.)	Urinary excretion (mg./24 hrs.)		Plasma 17-OH-CS‡ (μg./100 ml.)	Dosage of ACTH
			17-KS*	17-OH-CS†		
			27.2	19.6		
0	0	67	76.8		50–90	25 units i.v. over 8 hrs.
1	10	67	28.8	16.0		
2	10		32.4			
3	10		15.9	6.0		
			16.4	5.0		
4	10	67	26.8	5.6	30–42	25 units i.v. over 8 hrs.
5	4	67	12.4	5.0		
			12.1	3.2		
6	4	66	19.5	4.3	17–36	25 units i.v. over 8 hrs.
7	3		14.1	3.0		
8	3	65	10.0	2.4		
10	3	63	10.7	3.2		
12	3	61	10.8	3.6		
14	3	59	6.3	2.8		
			8.8	2.5		
16	3	59	10.8	6.0	17–32	25 units i.v. over 8 hrs.

* Normal, 6–12 mg.
† Normal, 2–10 mg.
‡ Normal, 5–20 μg.

during the study. Associated with the objective evidence of improvement there was a marked subjective response, with an increase in the sense of well-being and in libido.

During the seventh week of therapy a papular eruption developed over the chest and lower extremities. The rash, however, disappeared spontaneously five days later, although the drug was continued. Laboratory studies at this time showed normal values for the blood hemoglobin level, white blood cell, differential and platelet counts, urinalysis, cephalin flocculation test, and the levels of blood urea nitrogen, fasting blood sugar, serum electrolytes, total protein, albumin, globulin, alkaline phosphatase, bilirubin, and total cholesterol. Visual fields were normal and skeletal x-ray survey showed no change in the previously described osteoporosis. Examination of iliac bone marrow revealed no abnormalities. Glucose tolerance, however, showed considerable improvement over that in the previous study in June 1956 (Table 1).

Six weeks after discontinuation of o,p'DDD therapy, the excretion of

neutral 17-KS increased (Table 3) and the drug was restarted. The urinary 17-KS promptly returned to normal. The patient was then maintained in a state of clinical and biochemical remission with 2 Gm. of o,p'DDD daily. Side-reactions were minimal at this dosage level.[3]

DISCUSSION

Administration of o,p'DDD caused a significant decrease in adrenocortical function, as manifested by diminished urinary steroid excretion, improvement in glucose tolerance and a striking clinical response.

TABLE 3. URINARY 17-KS AND 17-OH-CS EXCRETION FOLLOWING DISCONTINUANCE AND LATER RE-INSTITUTION OF THERAPY WITH o,p'DDD

Time (wk.)	Weight of patient (Kg.)	Urinary excretion (mg./24 hrs.)		Status of o,p'DDD therapy
		17-KS*	17-OH-CS†	
1	57.5	9.7	3.2	Discontinued 1 wk. previously
2		9.3	2.4	
3		12.2	2.4	
4	56.8	7.4	5.3	
5		7.0	3.4	
6		18.2	5.2	
7	56.6	16.0	5.3	2 Gm. o,p'DDD per day re-instituted
8		11.8	5.5	

* Normal, 6–12 mg.
† Normal, 2–10 mg.

Our previous experience (6) with o,p'DDD demonstrated that this substance does not interfere with the chemical determination of urinary or plasma steroids. The observed changes in steroid levels can therefore be interpreted as true reflections of altered adrenocortical function. The possibility of a spontaneous remission appears most unlikely, since discontinuation of the medication was followed by a rise in the urinary excretion of neutral 17-ketosteroids, and the re-institution of therapy caused a prompt return to normal.

Results of the present study indicate that maintenance of effective suppression of the manifestations of Cushing's syndrome may be obtained

[3] Approximately nine months after institution of therapy (two months after submission of this paper), the patient died from a massive cerebral hemorrhage. Just prior to death the erythrocyte, leukocyte and platelet counts, and the coagulation mechanism were normal. Postmortem examination (Dr. A. Elguazabal) revealed adrenals of normal size without macroscopic or microscopic evidence of tumor, hemorrhage or necrosis. Except for cardiomegaly (left ventricular hypertrophy), atherosclerosis and a left intracerebral hemorrhage, no significant abnormalities were found in the other organs. Before the cerebral hemorrhage, the patient had been taking 1 Gm. of o,p'DDD daily. She had been active and working at her place of employment.

with considerably smaller doses than were employed initially. These smaller doses eliminate to a large extent the gastro-intestinal and central nervous system side-effects noted with larger amounts of the drug. The importance of using large dosages of the isomer during the early part of the treatment period needs to be established.

The available evidence suggests that o,p'DDD acts directly on the adrenal cortex rather than through the anterior pituitary (5). Moreover the findings of the present study indicate that the degree of adrenal responsiveness may be related to the daily amount of administered drug. Prolonged therapy over a period of sixteen weeks did not produce any evidence of hypoadrenalism. In a previous study by Sheehan *et al.* (12), failure to attain any clinical or biochemical changes with the use of technical DDD in a case of nontumorous Cushing's syndrome was most likely due to the low adrenal suppressive activity of the crude product (4) as well as to the small administered dose.

In view of our experience, o,p'DDD may prove to be a useful agent in the therapy of nontumorous Cushing's syndrome. It is still too early, however, to assess the long-term effects.

Addendum

Since the submission of this paper, 2 additional patients with Cushing's syndrome have been treated with o,p'-DDD by one of us (S.W.) and Drs. E. Wallace, J. Silverstein and L. Villadolid of the Medical Service of the State University of New York, College of Medicine at New York City. Early results indicate reduction of plasma and urinary steroid levels associated with improvement in some of the stigmata of Cushing's syndrome.

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Letters to the Editor

ANTITHYROID ANTIBODY IN JUVENILE DIABETICS*

To the Editor:

In the course of studies on the occurrence of antithyroid antibody in various thyroid disorders, such antibodies were demonstrated in the serum of 2 diabetic children with nontoxic goiter. This observation, and the reported association of thyrotoxicosis and juvenile diabetes (1) suggested that a more detailed survey of the occurrence of antithyroid antibody in juvenile diabetics would be of interest.

The sera of 58 diabetic children 16 years of age or less were tested by the Coons labeled-antibody method (2), an unfixed frozen section of normal human thyroid being treated with the patient's serum, and then

Table 1. Incidence of antithyroid antibody by Coon's labeled-antibody method

Series	Number	Positive	Per cent positive
Euthyroid nondiabetic children	178	2	1.1
Normal children (orphanage)	51	0	0
Pregnant women	100	3	3
Hyperthyroid children	14	10	71
Diabetic children	58	13	22

with fluorescein-labeled rabbit antihuman gamma globulin. The preparations were examined with a conventional microscope illuminated by a high-intensity mercury arc bulb. Significant selective staining of the cytoplasm but not of the nuclei of thyroid follicular epithelial cells was considered a positive reaction. Thirteen (22 per cent) of the sera of diabetic children showed a positive reaction for antithyroid antibody, and in another 10 the reaction was questionably or doubtfully positive. These values are compared in Table 1 to those for *a*) 178 euthyroid nondiabetic children studied over a period of several years by the metabolism laboratory, *b*) 51 presumably normal children in an orphanage, *c*) 100 unselected pregnant women, and *d*) 14 hyperthyroid children. The chi-square value of the data for diabetic children versus those for euthyroid nondiabetic

* These studies were supported in part by research Grants #A-998 and C-2143 from the National Institutes of Health, United States Public Health Service.

children is 33.2, indicative of a highly significant association of juvenile diabetes and antithyroid antibody. Studies carried out by this laboratory in conjunction with Dr. R. M. Blizzard and Dr. R. W. Chandler of the Columbus Children's Hospital (3) have shown that the labeled-antibody method of demonstrating antithyroid antibody yields results agreeing statistically with those obtained by the tanned red-cell agglutination or complement fixation methods, and that the Coons method demonstrates both agglutinating and complement-fixing antibodies.

A simple explanation of the observations described is that the biochemical dysfunction of the thyroid gland leading to autoimmunization is an inherent component of hereditary diabetes mellitus, but is far overshadowed clinically by dysfunction of the pancreatic islets. However, there are many possible ways in which the thyroid abnormality might be secondary to other aspects of diabetes mellitus, and further study will be necessary to elucidate the exact relationship between the two factors. A survey of the occurrence of antithyroid antibody in juvenile diabetics who have reached adult age, and in diabetics who did not develop the disease until adult life, is projected, as is analysis of the relation of maternal antithyroid antibody to the prognosis in infants of diabetic mothers.

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TRANSMETHYLATION OF 2-HYDROXYESTROGENS BY HUMAN TISSUE*

To The Editor:

In 1956 Axelrod, Miller and Herling (1) demonstrated the biologic 2-hydroxylation of steroids. In 1957 when 2-methoxyestrone was identified in human urine (2, 3), it became of interest to determine whether or not such 2-hydroxlated steroids were intermediates in this reaction. In a recent publication (4) it was shown that the intravenous administration of 2-hydroxyestradiol-17β to postmenopausal women resulted in a substantial urinary excretion of 2-methoxyestrone. Since both the liver and the kidney are known to be involved in intermediate steroid metabolism, and since the adrenal cortex is an active site of steroid hydroxylations, these tissues were selected for further study.

At various operations, 3 samples of liver weighing 0.9 to 1.2 Gm., 2 entire kidneys containing circumscribed neoplasms, and 1 normal adrenal weighing 4.1 Gm. were obtained. Samples of kidney tissue weighing 34 Gm. and 120 Gm. respectively, and an 85-Gm. sample of renal carcinoma were used in these experiments. Within thirty to forty-five minutes after interruption of the blood supply, the tissue was finely minced and was incubated in Krebs-Ringer phosphate-glucose buffer, pH 7.4 (1:2 w/v) together with DPN, TPN, DPNH and ATP. To this suspension was added 2-hydroxyestradiol-17β (1 mg. per 5 Gm. of tissue) (5) and *dl*-methionine-methyl-C^{14} (specific activity 20.8 μc. per mg.). Incubation was carried out at 37°C. for three hours in an atmosphere of 95 per cent O_2 and 5 per cent CO_2. The reaction was stopped with acetone, and a lipid extract was prepared by extraction with ethyl acetate (6). The residue after solvent evaporation was repeatedly chromatographed on paper in a methylcyclohexane-propylene glycol system (7) for various lengths of time.

The 3 liver incubations and the adrenal incubation yielded no detectable radioactive steroid zones upon chromatography and radioactive scanning. Contrariwise, both incubations of normal human kidney tissue showed 2 radioactive bands on the chromatograms, which reacted positively with ferric chloride-potassium ferricyanide reagent (8), and showed a deep pink fluorescence in ultraviolet light after treatment with fuming sulfuric acid (7). The radioactive areas were eluted and the residues crystallized from acetone-pentane. The more polar band yielded 4.3 mg. (m.p. 178–180°, uncorrected), and the less polar band yielded 1.2 mg. (m.p. 176–178°, uncorrected). The sulfuric-acid chromogen spectrum of the more polar compound (9) (determined on a Cary recording spectrophotometer) and the infrared spectrum in KBr (determined on a Perkin-

* This work was supported by grant A-1078, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service.

Elmer Model 21 infrared spectrometer) were identical with those of an authentic sample of 2-methoxyestradiol-17β; the less polar compound was confirmed as 2-methoxyestrone by the same criteria. Incubation of the renal carcinoma tissue yielded on the chromatogram a single radioactive band which was proved to be 2-methoxyestradiol-17β by the specified criteria.

Thus, under the conditions of these incubations, human liver and adrenal are unable to carry out the transmethylation reaction on 2-hydroxyestradiol-17β. On the other hand, kidney tissue carried out both transmethylation and oxidation of the 17-hydroxyl group. It is of considerable interest that malignant renal tissue retained the ability to transmethylate. It should be noted also that this is the first isolation of 2-methoxyestradiol-17β from human biologic material.

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Acknowledgment

We wish to acknowledge our indebtedness to Col. L. K. Mantell, Chief of Urology, Brooke General Hospital, for the surgical specimens. We also wish to thank Dr. Jack Fishman for the authentic samples of 2-methoxyestrone and 2-methoxyestradiol-17β.

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TRANSIENT MYXEDEMA

To the Editor:

The following case of myxedema seemed worth reporting because of some unusual features. Although the myxedema had been present for five years, and was not drug-induced, normal thyroid function was restored after four injections of thyroid-stimulating hormone (TSH).

A woman aged 27 was first seen in September 1958. During the previous five years she had become increasingly lethargic and had gained 20 pounds in weight. She was unduly sensitive to cold. Menstrual loss had been excessive for a year.

On examination she was obviously myxedematous. Her skin and hair were coarse and dry and the hands were cold. The eyebrows were generally thinned and there was puffiness of the skin under the eyes. Her voice was deep and hoarse. The ankle jerks were myoclonic. The thyroid gland was not palpable. There was normal development of

Table 1. Thyroid function tests beofre and after administration of TSH

Date	I^{131} test results*			Basal metabolic rate (%)	Serum cholesterol (mg./100 ml.)
	Urinary excretion, 0–48 hrs. (% of dose)	"T" index	Neck count at 48 hrs. (% of dose)		
Sept. 9, 1958	86	2.0	12	−18	426
TSH, 10 U.S.P. units daily for 4 days (Nov. 1–4, 1958)					
Nov. 4, 1959	45	9.6	51		412
Jan. 9, 1959	62	4.1	36		254
May 26, 1959					235
Nov. 17, 1959	54	7.8	39	+ 7	250

* Following oral administration of 1 μc. of I^{131}.

the breasts and sexual hair, and vaginal examination revealed a normal uterus and adnexae. The heart was not enlarged, the blood pressure was 120/90 mm.Hg, and the pulse was 52 beats per minute.

Laboratory investigation showed moderate anemia—hemoglobin level, 11.2 Gm. per 100 ml.; erythrocyte count, 3,500,000 per cu. mm.; and mean corpuscular volume, 100 cu.μ. The results of tests for thyroid function are listed in Table 1. The initial findings all confirmed the clinical diagnosis of myxedema; the 48-hour thyroidal I^{131} uptake was 12 per cent of the dose (1 μc.), with a 0–48 hour urinary excretion of 86 per cent, and a "T" index of 2.0 (1). The concentration of serum cholesterol was 426 mg. per 100 ml., and the basal metabolic rate was −18 per cent (Robertson and Reid standards) (2). The electrocardiogram showed low voltage with flat T waves. On x-ray examination, the chest and skull were normal.

A course of daily intramuscular injections of thyroid-stimulating hormone (Thytropar, Armour, 10 U.S.P. units) was given for four days, terminating on the day of the second radioiodine test (November 4, 1958), which showed a normal uptake.

The patient was not seen again until January 1959. She was a changed person.There was now no evidence of myxedema, and she was alert and active. She was not troubled by the winter cold. Her menstrual loss during November and December had been nor-

mal. The thyroidal I^{131} uptake was within the normal range and the serum cholesterol level had fallen to 254 mg. per 100 ml.

In March 1959, the patient was found to be pregnant (last menstrual period, January 9, 1959). She remained euthyroid throughout her pregnancy, and was delivered of a normal male child in October 1959.

In November 1959, the patient felt perfectly well and looked normal. Tests of thyroid function yielded normal results, and the electrocardiogram was normal. The hemoglobin level was 12.8 Gm. per 100 ml., and a blood film showed normal blood cell morphology.

Drug-induced myxedema has been observed following administration of iodine in cases of goiter (3, 4), as well as in initially nongoitrous patients (5). Recent reviews (6–9) stress the relative frequency of this condition. Among other chemical agents reported to have caused myxedema are cobalt (10), potassium chloride (11) and tolbutamide (12). Perrault and Klotz (13), in a review of iatrogenic myxedema, list other causes and suggest the possibility that patients may be exposed to such agents by accident as well as by design.

Myxedema induced by drug administration is often accompanied by goiter, and the fact that the present patient did not have a goiter when she was myxedematous may indicate that the disorder was not drug-induced. Further, a detailed history and examination excluded exposure, either accidental or therapeutic, to agents known to produce myxedema.

TSH was administered to ascertain whether the myxedema was primary or secondary (14, 15). Thus it was given as part of a diagnostic procedure, and the intention was to proceed with other tests of endocrine function. Following the administration of TSH we lost sight of the patient because she felt better. By the time she was seen in January 1959, she was obviously normal in all respects.

During the seven years of her married life she had remained barren despite the desire for a family, so it may be significant that she became pregnant two months after TSH was administered.

Selective pituitary failure or temporary thyroid failure must be postulated as the cause of her myxedema. In retrospect it is impossible to be more specific.

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ANDROGENIC EFFECTS OF A PROGESTATIONAL COMPOUND, 17α-ETHYNYL-19-NORTESTOSTERONE (NORLUTIN), ON THE HUMAN SEBACEOUS GLAND*

To the Editor:

Structurally, progesterone differs from testosterone only in the short side-chain at the 17 position of the D-ring. Biologically, progesterone has been converted to testosterone *in vitro* by rat testicular tissue (1), human ovarian tissue (2) and human interstitial-cell carcinoma tissue (3). Therefore, it is not surprising that agents with predominant progestational

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action may also have androgenic effects. Natural progesterone has been reported to have androgenic effects in animals, but only if massive unphysiologic amounts of the compound are administered (4–7). Even more striking has been the production of pseudohermaphrodism in human female infants born of mothers treated prenatally with 17α-ethynyl-19-nortestosterone (Norlutin) (8).

These serious developmental abnormalities have made it clear that progestational agents must be proved to be free of androgenic side-effects before clinical use. In the laboratory, androgenicity usually is evaluated by increase in weight of the prostate gland, seminal vesicles or testis of the rat. 17α-ethynyl-19-nortestosterone shows practically no androgenicity by these techniques (9); however, when the compound is given to pregnant rats, pseudohermaphrodism is produced in a significant number of the newborn females (10).

In the human subject, the sebaceous glands of the face, which are rudimentary in the prepuberal child, undergo massive enlargement when stimulated by androgens (11). This glandular enlargement represents a highly sensitive biologic system for demonstrating androgenic activity in humans; as little as 5 milligrams of methyltestosterone orally per day will produce adult-sized sebaceous glands in prepuberal subjects within two weeks (11). The following case histories illustrate 1) the androgenic activity of 17α-ethynyl-19-nortestosterone on the human sebaceous gland, and 2) the other signs of androgenicity produced in prepuberal boys given this compound.

CASE REPORTS

All 3 subjects were prepuberal male inmates of a school for mental defectives. All had infantile external genitalia, no pubic hair, non-oily skin, and rudimentary sebaceous glands, as seen in histologic sections of the cheek.[1] A daily dose of 20 mg. of 17α-ethynyl-19-nortestosterone[2] was given to each subject orally for a twelve-week period. This is approximately the dosage used therapeutically in adults.

1. E.B., aged 11. A small amount of pubic hair developed after three weeks of drug administration. By twelve weeks there was a pronounced growth of coarse black pubic hair. Biopsy specimens of the cheek at three and eight weeks showed enlargement of the sebaceous glands (Fig. 1). Oiliness was clinically apparent in eight weeks. Twelve weeks after starting administration of Norlutin, a small amount of axillary hair and a definite increase in mammary tissue could be observed. There was no increase in the size of the penis.

2. D.C., aged 7. Pubic hair developed after five weeks of therapy. Oiliness of the skin

[1] In all of our studies, attention was focused on the sebaceous glands of the face. The massive changes which occur in these glands during hormonal stimulation, as well as at puberty, make evaluation easier. Any changes in the smaller glands of areas such as the trunk and extremities are extremely difficult to determine.

[2] Norlutin®, supplied by Leon Warren, M.D., Parke, Davis & Company, Detroit, Michigan.

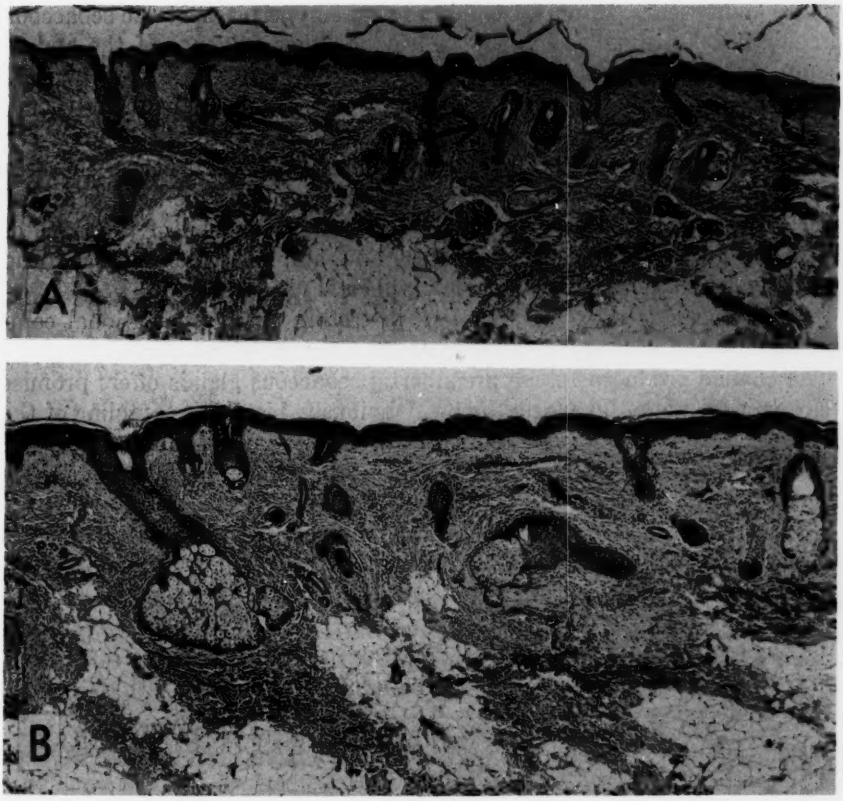

FIG. 1. Effect of the daily administration of 20 mg. of 17α-ethynyl-19-nortestosterone on the sebaceous glands of the cheek in an 11-year-old prepuberal male (Case 1). **A.** Control specimen. The sebaceous glands are barely discernible (indicated by arrows). **B.** After three weeks of therapy a biopsy specimen from the contralateral cheek shows marked glandular development. (Both A and B reduced from ×45.)

appeared in ten weeks. After twelve weeks, histologic sections showed definite hyperplasia of the sebaceous glands. In addition there was definite enlargement of the breasts.

3. J.T., aged 7. Pubic hair developed after eight weeks of therapy. Oiliness of the skin appeared at the same time, and there was histologic evidence of hyperplasia of the sebaceous glands at eight weeks. The breasts enlarged, but there was no increase in the size of the penis, and no development of axillary hair.

DISCUSSION

The interpretation of these data requires the knowledge that natural progesterone in approximately physiologic doses has no androgenic effect on the sebaceous glands of the prepuberal human subject. We have given 7 prepuberal children 50 mg. of progesterone intramuscularly daily for

six to eight weeks. In only 1 child did slight hyperplasia of the sebaceous glands develop; furthermore, there was no other evidence of the androgenic pubertal constellation (12).

Other studies have shown that sebaceous gland enlargement occurs earlier than either penile enlargement or the appearance of secondary sex hair (11). Undoubtedly, if biopsies had been performed earlier in these children, the changes in the sebaceous glands would have been observed earlier. In any event, the test period was short, and all of the signs of androgenicity were reversible. Short-term administration of the drug minimizes the danger of irreversible pubertal changes.

From the practical point of view, in testing new progestational compounds, the possibility of androgenicity must be ruled out in humans. The change produced in the prepubertal sebaceous glands offers promise as a test for potential androgenicity. Obviously, histologic sampling of the cheek is not a practical procedure. However, there is a simple means of determining sebaceous gland size by measuring sebum production. Sebum production is directly proportional to the size of the gland; therefore changes in size can be detected by the measurement of sebum. The technique itself, described elsewhere in detail, involves the gravimetric determination of lipid collected from the forehead for three hours on filter paper held in place with a rubber bandage (13). This procedure may offer an easy method of screening for androgenicity in a small number of human volunteers and is presently being investigated with this in mind.

SUMMARY

The sebaceous gland is a highly sensitive effector organ of androgenicity.

In 3 prepuberal male subjects given 20 mg. of 17α-ethynyl-19-nortestosterone daily, androgenic changes developed which were most consistently manifested by enlargement of the sebaceous glands.

The effect of progestional compounds on human prepuberal sebaceous glands may serve as a screening test for the determination of unwanted androgenic side-effects.

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Acknowledgment

We wish to thank the personnel of Woodbine State Colony, Woodbine, New Jersey (H. Von Bulow, Superintendent) for their cooperation in this study.

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HYPOPARATHYROIDISM REQUIRING MASSIVE AMOUNTS OF MEDICATION, WITH APPARENT RESPONSE TO MAGNESIUM SULFATE

To the Editor:

Hypoparathyroidism, whether primary or secondary, usually presents a relatively simple therapeutic problem. On rare occasions, however, it may present a difficult problem and may require massive amounts of medication. The following is a report of such a case:

CASE REPORT

Mrs. T.S. was first seen at the Jersey City Medical Center in July 1939 at the age of 34 years because of progressive enlargement of a mass in her neck associated with

irritability, palpitation on exertion, emotional lability and an 18-pound weight loss which had occurred in spite of a good appetite. The mass in her neck had been present for approximately fifteen years.

Physical examination showed a pulse rate of 80 per minute and a diffuse enlargement of the thyroid gland, especially of the left lobe. The basal metabolic rate (BMR) prior to hospitalization was +8 per cent and immediately after admission was −5 per cent.

Hospital course: On August 7, 1939, a subtotal thyroidectomy was performed under intratracheal oxygen-ether anesthesia. The operative procedure was apparently tolerated without difficulty. The evening of the operation a moderate amount of bleeding occurred at the operative site but there was no respiratory difficulty and no instrumentation of the operative site was required. On August 10, 1939, the third postoperative day, the patient complained of numbness in all extremities. These paresthesias continued until the time of discharge from the hospital on August 14, 1939. No further diagnostic studies were performed nor specific treatment given at that time. The pathologic report on the resected thyroid tissue was "nodular goiter with hyperinvolution."

She was readmitted to the hospital on August 17, 1939, complaining of numbness in her hands and feet and inability to flex her fingers. She also complained of persistent vague pains, weakness in her arms, legs, wrists, and hands and inability to walk because her "feet turned inward." She also complained of "electricity" throughout her entire body. Marked spasticity of the upper extremities, extreme flexion at the wrists, elbows and fingers and inversion of both feet were present, as were both Chvostek and Trousseau signs. The diagnosis of hypoparathyroidism was confirmed by chemical studies showing a serum calcium level of 5.2 mg. per 100 ml. and a serum phosphorus level of 4.0 mg. per 100 ml. (Table 1A).

In the subsequent five years, she received various medications including oral calcium salts, Viosterol, Percomorph oil, dihydrotachysterol, desiccated thyroid, low-phosphorus diet, occasional blood transfusions and occasional injections of Parathormone. She stated that she would seem to be free of symptoms for a short time when a new medication was started; then, while continuing the same medication the symptoms would return. On two occasions (August 1940 and December 1954) parathyroid gland transplantations were attempted without benefit. From 1942 until 1955, she required the administration of calcium gluconate intravenously every other day, and between 1944 and 1955 she received no other medication. During this period the concentration of serum calcium varied between 5.6 mg. and 9.0 mg. per 100 ml., and that of serum phosphorus between 7.6 mg. and 3.3 mg. per 100 ml. When an injection of calcium gluconate was omitted, or sometimes in spite of receiving this medication, frank tetany with convulsions and loss of consciousness would occur.

On October 31, 1955, oral therapy was again attempted, starting with 100,000 units of vitamin D daily. While taking this medication, the patient was able to discontinue the parenterally administered calcium until March 1956, at which time the Sulkowitch test indicated lack of calcium in the urine. Medication was then gradually increased so that by May 1956 the patient was receiving four capsules of dihydrotachysterol (total of 2.5 mg.), 800,000 units of Calciferol and 4 Gm. of calcium gluconate orally per day. At this dosage level the patient felt well and the urinary Sulkowitch reaction became strongly positive. Therefore, the dosage of medication was gradually reduced over a six-week period to a minimum of one capsule of dihydrotachysterol (0.625 mg.), 150,000 units of Calciferol and 4 Gm. of calcium gluconate orally per day. During this entire period and subsequently, the patient was instructed to use a low-phosphorus diet. The ingestion of alumina gels was not tolerated.

In August 1956, muscle cramps and carpal spasm recurred. Therefore the dosage of

TABLE 1 **A.** REPRESENTATIVE POST-THYROIDECTOMY BLOOD LEVELS (MG./100 ML.)

Date	Calcium	Phosphorus	Urea nitrogen	Glucose
Aug. 17, 1939	5.2	4.0	14.0	66
Aug. 14, 1940	6.9	5.6	17.0	82
Apr. 29, 1943	7.0	3.3	15	90
Dec. 12, 1955	9.0	7.7	—	—
Feb. 16, 1957	7.6	4.1	12.0	105
Jan. 14, 1959	9.7	5.3	—	—

B. ROENTGENOLOGIC, ELECTROENCEPHALOGRAPHIC AND ELECTROCARDIOGRAPHIC FINDINGS (1939–1957)

Roentgenograms

Chest: Normal on repeated examinations, 1939–1957.
Skull: Normal, Sept. 30, 1939.
Lower thoracic and lumbar spine: Calcification within a ligament adjacent to the anterosuperior border of the second lumbar vertebra on the left, Oct. 10, 1940.
Left hip: Minimal osteoarthritis, March 4, 1953.
Both hips: Osteoarthritis, Jan. 24, 1957.
Abdomen: Right kidney of normal size and shape; left kidney obscured by overlying intestinal contents; no evidence of metastatic calcification; blood vessels not calcified; Jan. 24, 1957.

Electroencephalogram (March 3, 1953)

EEG (12-lead) showed irregular pattern with moderate amount of medium-voltage, 7 per second activity in all areas and some low-voltage 18–22 per second activity mostly in the posterior and central leads. In response to satisfactory hyperventilation, there was some slowing and build-up; a single brief burst of mixed spiked waves and 5 per second activity developed synchronously. There were no focal signs. Impression: Abnormal tracing consistent with hypocalcemic state. The expected marked slowing did not occur during hyperventilation

Electrocardiogram (Jan. 23, 1957)

Rate: 53 per minute; P-R: 0.16 second; QRS: 0.08 second; Q-Tc: 0.58 second (normal 0.37 to 0.45 second at this rate). Impression: Tracing compatible with hypocalcemia.

medication was gradually increased again, so that by January 1957 she was taking a low-phosphorus diet, twelve capsules of dihydrotachysterol (7.25 mg.), 1.5 million units of Calciferol and thirty tablespoonfuls of calcium gluconate (approximately 100 Gm.) daily. From February 1957 to May 1958 she also received estrone (5 mg.) with testosterone (100 mg.) intramuscularly once monthly (1). While following this therapeutic regimen she felt relatively well. After being fairly comfortable for periods as long as two months, the patient would experience an exacerbation of muscle spasms requiring weekly intravenous injections of calcium gluconate for relief. When these symptoms developed, the urinary Sulkowitch reaction would be negative. However, there was no recurrence of the convulsions or loss of consciousness that occurred frequently while receiving parenteral calcium gluconate alone.

In May 1957, prednisone (40 mg. per day) was given for one week in addition to the other medications. There was no subjective or objective improvement and no increase in the output of urinary calcium as determined by the Sulkowitch test.

In May 1958, extensive kerototic folliculitis of the lower extremities developed. This condition failed to improve with the administration of oral antibiotics but did clear with reduction of vitamin D dosage to 300,000 units daily. With the reduced vitamin dosage there was no change in the patient's clinical condition, except for improvement and subsequent disappearance of the folliculitis.

In September 1958, magnesium sulfate in the form of Epsom salts was added to the previous regimen. The dosage was 1 teaspoonful three times a day (approximately 18 Gm.). At this time she was taking fourteen capsules (8.85 mg.) of dihydrotachysterol,

300,000 units of Calciferol, and thirty tablespoonfuls (approximately 100 Gm.) of calcium gluconate daily. Prior to the administration of magnesium sulfate, the patient had been somewhat constipated but with this therapy, she had one or two formed stools daily. There was no abdominal discomfort or diarrhea. With this routine, the patient became completely asymptomatic. In October 1958, the patient reduced her intake of calcium gluconate to ten tablespoonfuls (approximately 33 Gm.) daily. In March 1960, Trousseau's sign and mild muscle cramps developed, and the urinary Sulkowitch reaction for calcium was negative. Therefore she was advised to increase her intake of calcium gluconate to thirty tablespoonfuls (approximately 100 Gm.) daily. Since starting the oral magnesium sulfate, the patient felt better than she had in many years.

Representative post-thyroidectomy blood levels of calcium, phosphorus, urea nitrogen and glucose (1939–1957) are listed in Table 1A. A summary of the roentgenographic, electroencephalographic and electrocardiographic findings (1939–1957) is presented in Table 1B.

DISCUSSION

We were able to find only one report (2) of a patient requiring approximately the amount of medication this patient received. The reported case, hypoparathyroidism also developed postoperatively and also was not adequately controlled in spite of the use of massive amounts of medication. The reason for the incomplete response to such massive therapy can only be hypothesized. The development of a keratotic folliculitis of the lower extremities which did not respond to antibiotics but did respond promptly to reduction of the vitamin D intake, strongly suggests that there was no defect in the absorption of vitamin D. Since absorption of vitamin D apparently was not defective, it is not likely that absorption of dihydrotachysterol was defective. However, it is certain that the absorption of calcium was inadequate, as manifested by the persistent lack of calcium in the urine (Sulkowitch reagent) and the low level of calcium in the serum (7.6 mg. per 100 ml.) when tested during the time of massive medication.

In view of the possibility that a block or defect of the gastro-intestinal mucosa led to failure of absorption of calcium, or that some sort of antigen-antibody response caused loss of effectiveness of vitamin D or dihydrotachysterol after the initial response, a trial course of prednisone was administered in what is usually considered adequate dosage. This was totally without clinical benefit to the patient.

Hirschfelder and Haury (3) reported 2 patients with hypoparathyroidism, muscular twitching and a low concentration of plasma magnesium. However, these patients did not receive magnesium therapy and no comment was made regarding their clinical course. Potts and Roberts (4) described 2 patients in whom neurologic symptoms developed immediately after surgical removal of parathyroid adenomas for hyperparathyroidism. In these cases, a low serum magnesium level was the only demonstrable electrolyte abnormality. Both patients appeared to improve dramatically

in association with the administration of magnesium salts parenterally. Potts and Roberts suggested that the development of neurologic symptoms in the immediate postoperative period after removal of a parathyroid adenoma is due to retention of magnesium. This explanation would not be applicable to the patient presented here, since she had undergone surgery twenty years earlier for a nontoxic nodular goiter. However, these reports do indicate that a relationship exists between magnesium metabolism and parathyroid function. Magnesium deficiency in itself will cause neuromuscular irritability manifested by hyperexcitability and convulsions. It would appear that, in the present patient, the administration of oral magnesium not only corrected a deficiency of magnesium itself, but also improved the absorption and utilization of calcium. The improvement in calcium metabolism was indicated by the serum calcium level of 9.7 mg. per 100 ml. and the presence of calcium in the urine even after the intake of vitamin D was sharply reduced.

SUMMARY

A patient with hypoparathyroidism secondary to surgical resection of a nontoxic nodular goiter required massive dosages of standard medications (calcium, dihydrotachysterol, vitamin D) and a low-phosphorus diet plus occasional supplements of calcium salts parenterally. The oral administration of magnesium salts was accompanied by prompt amelioration of symptoms. The possible mechanisms are discussed.

LeRoy Homer, M.D.

150 Green Street,
Woodbridge, New Jersey,
June 11, 1958

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The Endocrine Society

The 1961 Annual Meeting

The Forty-Third Annual Meeting of the Endocrine Society will be held in the Hotel Biltmore, New York, N. Y., Thursday, Friday and Saturday, *June 22, 23 and 24, 1961.*

The Scientific Sessions will be held from 9:00 a.m. to 5:00 p.m. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 23, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make their hotel reservations immediately. The Biltmore will hold 300 bedrooms for members until May 1, 1961, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 26 for the American Medical Association and other meetings, it is imperative that your reservations be made from June 26 on, directly through the A. M. A. Housing Bureau. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1961.

Final program, membership card and advance registration forms will be sent on May 1, 1961 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send an original and four copies of the title and abstract to the Vice-President, Dr. Roy Hertz, National Cancer Institute, Bethesda, Maryland, not later than *February 15, 1961.* It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that those will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately 200 abstracts submitted each year and compiling of the program from these involves a tremendous amount of time and effort on the part of the Program Committee.

THE COUNCIL REQUESTS THAT AUTHORS ADHERE STRICTLY TO THE FOLLOWING RULES WHEN SUBMITTING ABSTRACTS; OTHERWISE THEY CANNOT BE CONSIDERED:

1. IT IS ASSUMED THAT ABSTRACTS SUBMITTED FOR THIS PROGRAM HAVE NOT AND WILL NOT BE SUBMITTED ELSEWHERE.
2. Abstracts may not exceed 200 words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulae cannot be used.
3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed 15 words;
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. Authors who wish receipt of their abstracts acknowledged should enclose self-addressed post card with the title of the abstract noted thereon.
5. Authors are also to enclose 3×5 inch filing cards, one for each author, giving his name and initials in the upper right-hand corner, and the complete title of the abstract with all the authors listed in the center of the card. There should be a second card enclosed listing the title and all authors, and the mailing address of the senior author. This is necessary for indexing. Senior authors will be notified of the acceptance of their papers for the final program.
6. The body of the abstract, typed double space, should follow the heading. The original copy should be on bond paper. The original and four copies should be forwarded to Dr. Hertz.
7. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

The 1961 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors. Endocrine Society awards are limited to citizens of the United States and Canada.

FRED CONRAD KOCH AWARD

In 1957 a substantial legacy was bequeathed to the Society by the late

Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle and in 1960 was presented to Dr. Emil Witschi. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954.

The Ciba Award

The Ciba Award is given in recognition of the meritorious accomplishments of an investigator in the field of basic or clinical endocrinology who has not reached his thirty-sixth birthday before June 1 of the year in which the Award is presented. This Award was established in 1942 and first presented in 1944. The Award is presently in the amount of $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

The American Goiter Association

The next Annual Meeting of the American Goiter Association will be held in the Warwick Hotel, Philadelphia, Pennsylvania, *May 3–6, 1961.* Arrangements are being made for a Clinical Day in the Philadelphia Hospitals on May 3. Scientific papers will be presented in the Warwick Hotel on May 4, 5 and 6. For further information address the Secretary, John C. McClintock, M.D., 702 Madison Avenue, Albany 8, N.Y.

1961 Laurentian Hormone Conference

The 1961 Laurentian Hormone Conference of the AAAS will be held at Hoberg's Resort, Lake County, California, during the week *September 4–9*. Investigators interested in attending this Conference should make application to the Committee on Arrangement of the Laurentian Hormone Conference, 222 Maple Ave., Shrewsbury, Massachusetts, at an early date and in any event *no later than May 10, 1961*. A conference rate of $14.50 per day per person is extended to all invited participants, with reduced rates for children. Since the number of our participants is necessarily limited by available accommodations, all applications are screened and invitations to attend are issued by the Committee on Arrangements by the second week in June. Invitations issued to applicants will include family members and indication of the number of such members should be made upon application to the Committee. The following program has been arranged.

PROGRAM

I. Hormonal Polypeptides and Their Functional Activities

Isolation, structure, synthesis and biological activities of adrenocorticotropins (ACTH).
C. H. Li, University of California, Berkeley.
Monday evening, September 4.

Synthetic pituitary hormones.
Klaus Hofman and Haruaki Yajima, University of Pittsburgh School of Medicine, Pittsburgh.
Tuesday morning, September 5.

Studies on a lipid-mobilizing substance of the pituitary gland.
Daniel Rudman, Robert L. Hirsch, and Forrest E. Kendall, Columbia University Research Service, Goldwater Memorial Hospital, and Departments of Medicine, Pathology and Biochemistry, College of Physicians & Surgeons, New York.
Tuesday morning, September 5.

Normal and abnormal regulation of corticotropin secretion in man.
Grant W. Liddle, Donald Island, and Clifton Meader, Vanderbilt University, Nashville.
Tuesday evening, September 5.

Angiotensin, a renal hormone.
Irving H. Page and F. M. Bumpus, The Cleveland Clinic, Cleveland.
Wednesday morning, September 6.

II. Thyroid Hormones

The natural occurrence of goitrogenic agents.
Monte A. Greer, University of Oregon School of Medicine, Portland
Wednesday morning, September 6.

Some extracellular and intracellular mechanisms governing the utilization of thyroid hormones.
Jamshed R. Tata, Wenner-Grens Institute, Stockholm, Sweden.
Wednesday evening, September 6.

III. Parathyroid Hormone

The chemistry, biochemistry, and physiology of the parathyroid hormone.
Howard Rasmussen, Rockefeller Institute for Medical Research, New York.
Thursday morning, September 7.

Clinical endocrinology of parathyroid hormone excess.
Gilbert S. Gordan, Eugene Eisenberg and H. F. Loken, University of California Medical Center, San Francisco
Thursday morning, September 7.

IV. Steroid Hormones

Methods for measuring estrogens and their application to problems in gynecology and obstetrics.
J. B. Brown and G. D. Matthew, Clinical Endocrinology Research Unit, Edinburgh, Scotland.
Friday morning, September 8.

Some studies of the mechanism of estrogen action.
Elwood V. Jensen, Ben May Laboratories, University of Chicago, Chicago.
Friday morning, September 8.

Anti-estrogens.
C. W. Emmens, Department of Veterinary Physiology, Sydney, Australia.
Friday evening, September 8.

The use of cortisone to condition hosts for successful tissue grafting.
Helene Wallace Toolan, Sloan-Kettering Institute for Cancer Research, New York.
Saturday morning, September 9.

A comparison of the mechanisms of hormonal and substrate induction of rat liver tryptophan pyrrolase.
Philip Feigelson, M. Feigelson and O. Greengard, Columbia University Institute of Cancer Research, New York.
Saturday morning, September 9.

New York University Post-Graduate Medical School

announces post-graduate course 563R in

GYNECOLOGICAL ENDOCRINOLOGY

A practical, didactic and clinical presentation with emphasis on the therapeutic management of endocrine disorders in the female, including a discussion of the diagnosis and management of intersex, will be offered. Endocrine therapy for menstrual abnormalities and ovulatory defects will be presented. Adrenocortical, thyroid, ovarian and pituitary abnormalities will be emphasized. Practical diagnostic tests are described and demonstrated. The therapeutic use of the newer progestational, gonadal and corticoid steroids will be discussed, as well as available pituitary preparations. Some pertinent aspects of male endocrinology will be included. Five-day, full-time course, *April 17 through April 21, 1961*. Tuition: $140.00. Given under the direction of Dr. Herbert S. Kupperman. For further information: Office of the Associate Dean, New York University Post-Graduate Medical School, 550 First Avenue, New York 16, N. Y.

Book Register

Abstracts of Japanese Medicine. Abstracts of the medical literature of Japan in the clinical and theoretical fields, covering some 500 current Japanese medical periodicals not usually accessible to Western readers. This service of Excerpta Medica is valuable to those wishing to follow the progress of Japanese medicine. Edited by T. YOSHIDA, Tokyo and J. HUIZINGA, Amsterdam. Aided by a grant from The Public Health Service, National Institutes of Health. Annual subscription rate, U.S.A., $30.00. Excerpta Medica Foundation, 119 Herengracht, Amsterdam, The Netherlands.

ACTH. Proceedings of the Sheffield Corticotropin Conference. Edited by DR. H. F. WEST. Thirty-one participants attended the Conference which was held at the University of Sheffield, January 7 and 8, 1960. The papers presented and the discussions included: The Nature of Corticotropins and Their Relation to M.S.H.; The Bioassay of Corticotropins; The Use of Urinary Steroid Assays to Determine the Effect of Corticotropin in Clinical Practice; Corticotropin and Aldosterone Secretion; The Effect of ACTH on the Human Adrenal Cortex; The Action of Corticotropin on Enzymes of the Adrenal Cortex in Man; Acquired Resistance to Corticotropin; Diagnostic Use of Corticotropin; Corticosteroids and Corticotropin in the Treatment of Pulmonary Tuberculosis; and The Comparison of Long Term Corticotropin and Oral Corticosteroid Therapy in the Treatment of Rheumatoid Arthritis; paper; 135 pages; 1960. (Ferring AB, Malmö, Sweden.) Munksgaard, Copenhagen, Denmark.

Background Material for the Development of Radiation Protection Standards. Report No. 1, Federal Radiation Council. Recommendations of the Council so far approved by the President provide numerical values for radiation protection guides for the whole body and certain organs of radiation workers and for the whole body of persons in the general population, as well as an average population gonadal dose. Research needs are listed. Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. Price 30 cents.

Ciba Foundation Study Group. No. 5. Regulation of the Inorganic Ion Content of the Cells. In honor of PROF. E. J. CONWAY, Department of Biochemistry and Pharmacology, University College, Dublin. Edited by G. E. W. WOLSTENHOLME, O.B.E., M.R.C.P. and C. M. O'CONNOR, B.Sc. for the Ciba Foundation. Twenty-three participants in a study group (April 6, 1960) discuss the critical energy barriers to active transport in muscle and the redox pump theory, osmotic regulation and ion transport of the frog skin epithelium, changes in the passive cation permeability of erythrocytes in low electrolyte media, factors determining the inorganic ion content of yeast cells, the part played by phosphate compounds in the regulation of certain inorganic ions in cells, and the effect of complete and partial inhibition of metabolism on active transport in nerve and muscle; 100 pages; 16 illustrations; 1960. Little, Brown and Company, Boston, Mass.